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(54) Title: EXTRACELLULAR SIGNAL-REGULATED KINASE, SEQUENCES, AND METHODS OF PRODUCTION AND USE		
(57) Abstract		
<p>The present invention relates, in general, to an extracellular signal regulated kinase, ERK-5. In particular, the present invention relates to nucleic acid molecules coding for ERK-5; ERK-5 polypeptides; recombinant nucleic acid molecules; cells containing the recombinant nucleic acid molecules; antisense ERK-5 nucleic acid constructs; antibodies having binding affinity to an ERK-5 polypeptide; hybridomas containing the antibodies; nucleic acid probes for the detection of ERK-5 nucleic acid; a method of detecting ERK-5 nucleic acid or polypeptide in a sample; kits containing nucleic acid probes or antibodies; a method of detecting a compound capable of binding to ERK-5 or a fragment thereof; a method of detecting an agonist or antagonist of ERK-5 activity; a method of agonizing or antagonizing ERK-5 associated activity in a mammal; a method of treating diabetes mellitus, skeletal muscle diseases, Alzheimer's disease, or peripheral neuropathies in a mammal with an agonist or antagonist of ERK-5 activity; and a pharmaceutical composition comprising an ERK-5 agonist or antagonist.</p>		

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DESCRIPTIONExtracellular Signal-Regulated Kinase, Sequences, and
Methods of Production and UseBackground of the InventionField of the Invention

The present invention relates, in general, to an extracellular signal regulated kinase, ERK-5. In particular, the present invention relates to nucleic acid molecules coding for ERK-5; ERK-5 polypeptides; recombinant nucleic acid molecules; cells containing the recombinant nucleic acid molecules; antisense ERK-5 nucleic acid constructs; antibodies having binding affinity to an ERK-5 polypeptide; hybridomas containing the antibodies; nucleic acid probes for the detection of ERK-5 nucleic acid; a method of detecting ERK-5 nucleic acid or polypeptide in a sample; kits containing nucleic acid probes or antibodies; a method of detecting a compound capable of binding to ERK-5 or a fragment thereof; a method of detecting an agonist or antagonist of ERK-5 activity; a method of agonizing or antagonizing ERK-5 associated activity in a mammal; a method of treating diabetes mellitus, skeletal muscle diseases, Alzheimer's disease, or peripheral neuropathies in a mammal with an agonist or antagonist of ERK-5 activity; and a pharmaceutical composition comprising an ERK-5 agonist or antagonist.

Background Information

Phosphorylation of serine/threonine residues on ribosomal protein S6 kinases (Ballou et al., J. Biol. Chem. 263:1188-1194(1988)), phosphatase 1 G binding protein (Dent et al., Nature 348:302-308 (1990)), and acetyl coA-carboxylase (Borthwick et al., Biochem J. 270:795-801 (1990)) occur in response to insulin and other extracellular cues. Ray and Sturgill, Proc. Natl. Acad.

Sci. USA 84:1502-1506 (1987), Cicciirelli et al., J. Biol. Chem. 263:2009-2019 (1988), and Hoshi et al., J. Biol. Chem. 263:5396-5401 (1988) have identified a micro tubule-associated protein 2 (MAP2)/myelin basic protein
5 (MBP) kinase that in response to insulin contains phosphate on serine/threonine residues (Ray and Sturgill, Proc. Natl. Acad. Sci. USA 85:3753-3757 (1988); Boulton et al., Cell 65:663-675 (1991)). Ribosomal protein S6 kinase has been identified as one potential target for this
10 kinase (Sturgill et al., Nature 334:715-718 (1988); Gregory et al., J. Biol. Chem. 264:18397-18401 (1989); Ahn and Krebs, J. Biol. Chem. 265:11495-11591 (1990)). Boulton et al., Biochemistry 30:278-286 (1991) and Boulton et al., Science 249:64-65 (1990) describe the purification
15 and cloning of a MAP2/MBP kinase which they named extracellular signal-regulated kinase 1 (ERK-1). Using probes derived from ERK-1, two novel kinases were identified, ERK-2 and ERK-3 (Boulton and Cobb, Cell Regulation 2:357-371 (May 1991); Boulton et al., Cell
20 65:663-675 (May 17, 1991)). A fourth ERK has been briefly described (Cobb et al., Cell Regulation 2:965-978 (Dec. 1991) and WO 91/19008 published December 12, 1991).

The present invention provides a novel ERK, ERK-5. ERK-5 shows 61% similarity (38% identity) to the human
25 ERK1 peptide sequence, 64% similarity to the rat ERK1 and ERK2 (39% and 37% identity, respectively) and 55% similarity to the rat ERK3 (30% identity).

Summary of the Invention

The invention provides ERK-5.

30 The invention also provides an isolated nucleic acid molecule coding for a polypeptide comprising an amino acid sequence corresponding to ERK-5, or at least 9 contiguous amino acids thereof.

The invention further provides a substantially pure
35 polypeptide comprising an amino acid sequence

corresponding to ERK-5, or at least 9 contiguous amino acids thereof.

The invention also provides a nucleic acid probe for the detection of the presence of ERK-5 in a sample.

5 The invention further provides a method of detecting ERK-5 RNA in a sample.

The invention also provides a kit for detecting the presence of ERK-5 RNA in a sample.

10 The invention further provides a recombinant nucleic acid molecule comprising, 5' to 3', a promoter effective to initiate transcription in a host cell and the above-described isolated nucleic acid molecule.

The invention also provides a recombinant nucleic acid molecule comprising a vector and the above-described
15 isolated nucleic acid molecule.

The invention further provides a recombinant nucleic acid molecule comprising a transcriptional region functional in a cell, a sequence complimentary to an RNA sequence encoding an amino acid sequence corresponding to
20 the above-described polypeptide, and a transcriptional termination region functional in said cell.

The invention also provides a cell that contains the above-described recombinant nucleic acid molecule.

The invention further provides an organism that
25 contains the above-described recombinant nucleic acid molecule.

The invention also provides an antibody having binding affinity to an ERK-5 polypeptide, or a binding fragment thereof.

30 The invention further provides a method of detecting an ERK-5 polypeptide in a sample.

The invention also provides a method of measuring the amount of ERK-5 in a sample.

The invention further provides a diagnostic kit
35 comprising a first container means containing the above-described antibody, and a second container means

containing a conjugate comprising a binding partner of said monoclonal antibody and a label.

The invention also provides a hybridoma which produces the above-described monoclonal antibody.

5 The invention further provides a method of detecting a compound capable of binding to ERK-5 or a fragment thereof.

The invention also provides a method of detecting an agonist or antagonist of ERK-5 activity.

10 The invention further provides a method of agonizing or antagonizing ERK-5 associated activity in a mammal.

The invention also provides a method of treating diabetes mellitus, skeletal muscle diseases, Alzheimer's disease, or peripheral neuropathies in a mammal with an
15 agonist or antagonist of ERK-5 activity.

The invention further provides a pharmaceutical composition comprising an ERK-5 agonist or antagonist.

Further objects and advantages of the present invention will be clear from the description that follows.

20 Brief Description of the Drawings

Figure 1. Nucleotide (SEQ ID NO: 1) and amino acid sequence (SEQ ID NO:2) of hERK5.

Figure 2. Northern blot of brain, liver, placenta, muscle, spleen, kidney, duodenum, and stomach RNAs.

25 Figure 3. hERK5-GST fusion protein expression in E. coli (Coomassie Blue Staining).

Figure 4. Western blot of total lysates from E. coli expressing hERK5-GST fusion protein or GST protein (control).

30 Figure 5. Expression of hERK5 in 293 cells. Lane 1: 293 cells transfected with pcDNA1 vector. Lane 2: 293 cells transfected with hERK5-pcDNA1 construct.

Figure 6. Stable cell lines expressing hERK5. Lane 1: untransfected NIH 3T3 cells. Lanes 2-5: NIH 3T3 cell
35 clones transfected with the cvn-hERK-5 construct growing in the presence of 100 nM methotrexate. Lane 6: Cell

clone of lane 5, 100 nM methotrexate. Lanes 7-8: Cell clone of lane 5, 200 nM methotrexate. Lane 9: Cell clone of lane 5, 500 nM methotrexate. Lane 10: Cell lysate of 293 cells transfected with pcDNAI-hERK-5 construct.

5 Definitions

In the description that follows, a number of terms used in recombinant DNA (rDNA) technology are extensively utilized. In order to provide a clear and consistent understanding of the specification and claims, including
10 the scope to be given such terms, the following definitions are provided.

Isolated Nucleic Acid Molecule. An "isolated nucleic acid molecule", as is generally understood and used herein, refers to a polymer of nucleotides, and includes
15 but should not be limited to DNA and RNA.

DNA Segment. A DNA segment, as is generally understood and used herein, refers to a molecule comprising a linear stretch of nucleotides wherein the nucleotides are present in a sequence that may encode,
20 through the genetic code, a molecule comprising a linear sequence of amino acid residues that is referred to as a protein, a protein fragment or a polypeptide.

Gene. A DNA sequence related to a single polypeptide chain or protein, and as used herein includes the 5' and
25 3' untranslated ends. The polypeptide can be encoded by a full-length sequence or any portion of the coding sequence, so long as the functional activity of the protein is retained.

Complementary DNA (cDNA). Recombinant nucleic acid
30 molecules synthesized by reverse transcription of messenger RNA ("mRNA").

Structural Gene. A DNA sequence that is transcribed into mRNA that is then translated into a sequence of amino acids characteristic of a specific polypeptide.

35 Restriction Endonuclease. A restriction endonuclease (also restriction enzyme) is an enzyme that has the

capacity to recognize a specific base sequence (usually 4, 5, or 6 base pairs in length) in a DNA molecule, and to cleave the DNA molecule at every place where this sequence appears. For example, EcoRI recognizes the base sequence
5 GAATTC/CTTAAG.

Restriction Fragment. The DNA molecules produced by digestion with a restriction endonuclease are referred to as restriction fragments. Any given genome may be digested by a particular restriction endonuclease into a discrete
10 set of restriction fragments.

Agarose Gel Electrophoresis. To detect a polymorphism in the length of restriction fragments, an analytical method for fractionating double-stranded DNA molecules on the basis of size is required. The most
15 commonly used technique (though not the only one) for achieving such a fractionation is agarose gel electrophoresis. The principle of this method is that DNA molecules migrate through the gel as though it were a sieve that retards the movement of the largest molecules
20 to the greatest extent and the movement of the smallest molecules to the least extent. Note that the smaller the DNA fragment, the greater the mobility under electrophoresis in the agarose gel.

The DNA fragments fractionated by agarose gel
25 electrophoresis can be visualized directly by a staining procedure if the number of fragments included in the pattern is small. The DNA fragments of genomes can be visualized successfully. However, most genomes, including the human genome, contain far too many DNA sequences to
30 produce a simple pattern of restriction fragments. For example, the human genome is digested into approximately 1,000,000 different DNA fragments by EcoRI. In order to visualize a small subset of these fragments, a methodology referred to as the Southern hybridization procedure can be
35 applied.

Southern Transfer Procedure. The purpose of the Southern transfer procedure (also referred to as blotting)

is to physically transfer DNA fractionated by agarose gel electrophoresis onto a nitrocellulose filter paper or another appropriate surface or method, while retaining the relative positions of DNA fragments resulting from the
5 fractionation procedure. The methodology used to accomplish the transfer from agarose gel to nitrocellulose involves drawing the DNA from the gel into the nitrocellulose paper by capillary action.

Nucleic Acid Hybridization. Nucleic acid
10 hybridization depends on the principle that two single-stranded nucleic acid molecules that have complementary base sequences will reform the thermodynamically favored double-stranded structure if they are mixed under the proper conditions. The double-stranded structure will be
15 formed between two complementary single-stranded nucleic acids even if one is immobilized on a nitrocellulose filter. In the Southern hybridization procedure, the latter situation occurs. As noted previously, the DNA of the individual to be tested is digested with a restriction
20 endonuclease, fractionated by agarose gel electrophoresis, converted to the single-stranded form, and transferred to nitrocellulose paper, making it available for reannealing to the hybridization probe.

Hybridization Probe. To visualize a particular DNA
25 sequence in the Southern hybridization procedure, a labeled DNA molecule or hybridization probe is reacted to the fractionated DNA bound to the nitrocellulose filter. The areas on the filter that carry DNA sequences complementary to the labeled DNA probe become labeled
30 themselves as a consequence of the reannealing reaction. The areas of the filter that exhibit such labeling are visualized. The hybridization probe is generally produced by molecular cloning of a specific DNA sequence.

Oligonucleotide or Oligomer. A molecule comprised of
35 two or more deoxyribonucleotides or ribonucleotides, preferably more than three. Its exact size will depend on many factors, which in turn depend on the ultimate

function or use of the oligonucleotide. An oligonucleotide may be derived synthetically or by cloning.

Sequence Amplification. A method for generating large amounts of a target sequence. In general, one or
5 more amplification primers are annealed to a nucleic acid sequence. Using appropriate enzymes, sequences found adjacent to, or in between the primers are amplified.

Amplification Primer. An oligonucleotide which is capable of annealing adjacent to a target sequence and
10 serving as an initiation point for DNA synthesis when placed under conditions in which synthesis of a primer extension product which is complementary to a nucleic acid strand is initiated.

Vector. A plasmid or phage DNA or other DNA sequence
15 into which DNA may be inserted to be cloned. The vector may replicate autonomously in a host cell, and may be further characterized by one or a small number of endonuclease recognition sites at which such DNA sequences may be cut in a determinable fashion and into which DNA
20 may be inserted. The vector may further contain a marker suitable for use in the identification of cells transformed with the vector. Markers, for example, are tetracycline resistance or ampicillin resistance. The words "cloning vehicle" are sometimes used for "vector."

25 Expression. Expression is the process by which a structural gene produces a polypeptide. It involves transcription of the gene into mRNA, and the translation of such mRNA into polypeptide(s).

Expression Vector. A vector or vehicle similar to a
30 cloning vector but which is capable of expressing a gene which has been cloned into it, after transformation into a host. The cloned gene is usually placed under the control of (i.e., operably linked to) certain control sequences such as promoter sequences.

35 Expression control sequences will vary depending on whether the vector is designed to express the operably linked gene in a prokaryotic or eukaryotic host and may

additionally contain transcriptional elements such as enhancer elements, termination sequences, tissue-specificity elements, and/or translational initiation and termination sites.

5 Functional Derivative. A "functional derivative" of a sequence, either protein or nucleic acid, is a molecule that possesses a biological activity (either functional or structural) that is substantially similar to a biological activity of the protein or nucleic acid sequence. A
10 functional derivative of a protein may or may not contain post-translational modifications such as covalently linked carbohydrate, depending on the necessity of such modifications for the performance of a specific function. The term "functional derivative" is intended to include
15 the "fragments," "segments," "variants," "analogs," or "chemical derivatives" of a molecule.

As used herein, a molecule is said to be a "chemical derivative" of another molecule when it contains additional chemical moieties not normally a part of the
20 molecule. Such moieties may improve the molecule's solubility, absorption, biological half life, and the like. The moieties may alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, and the like. Moieties
25 capable of mediating such effects are disclosed in Remington's Pharmaceutical Sciences (1980). Procedures for coupling such moieties to a molecule are well known in the art.

Fragment. A "fragment" of a molecule such as a
30 protein or nucleic acid is meant to refer to any portion of the amino acid or nucleotide genetic sequence.

Variant. A "variant" of a protein or nucleic acid is meant to refer to a molecule substantially similar in structure and biological activity to either the protein or
35 nucleic acid, or to a fragment thereof. Thus, provided that two molecules possess a common activity and may substitute for each other, they are considered variants as

that term is used herein even if the composition or secondary, tertiary, or quaternary structure of one of the molecules is not identical to that found in the other, or if the amino acid or nucleotide sequence is not identical.

5 Analog. An "analog" of a protein or genetic sequence is meant to refer to a protein or genetic sequence substantially similar in function to a protein or genetic sequence described herein.

10 Allele. An "allele" is an alternative form of a gene occupying a given locus on the chromosome.

15 Mutation. A "mutation" is any detectable change in the genetic material which may be transmitted to daughter cells and possibly even to succeeding generations giving rise to mutant cells or mutant individuals. If the descendants of a mutant cell give rise only to somatic cells in multicellular organisms, a mutant spot or area of cells arises. Mutations in the germ line of sexually reproducing organisms may be transmitted by the gametes to the next generation resulting in an individual with the new mutant condition in both its somatic and germ cells. A mutation may be any (or a combination of) detectable, unnatural change affecting the chemical or physical constitution, mutability, replication, phenotypic function, or recombination of one or more deoxyribonucleotides; nucleotides may be added, deleted, substituted for, inverted, or transposed to new positions with and without inversion. Mutations may occur spontaneously and can be induced experimentally by application of mutagens. A mutant variation of a nucleic acid molecule results from a mutation. A mutant polypeptide may result from a mutant nucleic acid molecule.

30 Species. A "species" is a group of actually or potentially interbreeding natural populations. A species variation within a nucleic acid molecule or protein is a change in the nucleic acid or amino acid sequence that

occurs among species and may be determined by DNA sequencing of the molecule in question.

Substantially Pure. A "substantially pure" protein or nucleic acid is a protein or nucleic acid preparation
5 that is generally lacking in other cellular components.

Detailed Description of the Invention

I. Isolated Nucleic Acid Molecules Coding for ERK-5 Polypeptides, and Fragments Thereof.

In one embodiment, the present invention relates to
10 an isolated nucleic acid molecule coding for a polypeptide having an amino acid sequence corresponding to ERK-5, or at least 9 contiguous amino acids thereof (preferably, at least 10, 15, 20, or 30 contiguous amino acids thereof). In one preferred embodiment, the isolated nucleic acid
15 molecule comprises the sequences set forth in SEQ ID NO: 1; allelic, mutant or species variation thereof, or at least 27 contiguous nucleotides thereof (preferably at least 30, 35, 40, or 50 contiguous nucleotides thereof). In another preferred embodiment, the isolated nucleic acid
20 molecule encodes the amino acid sequence set forth in SEQ ID NO:2, or mutant or species variation thereof, or at least 9 contiguous amino acids thereof (preferably, at least 10, 15, 20, or 30 contiguous amino acids thereof).

Also included within the scope of this invention are
25 the functional equivalents of the herein-described isolated nucleic acid molecules. The degeneracy of the genetic code permits substitution of certain codons by other codons which specify the same amino acid and hence would give rise to the same protein. The nucleic acid
30 sequence can vary substantially since, with the exception of methionine and tryptophan, the known amino acids can be coded for by more than one codon. Thus, portions or all of the ERK-5 gene could be synthesized to give a nucleic acid sequence significantly different from that shown in SEQ ID
35 NO: 1. The encoded amino acid sequence thereof would, however, be preserved.

In addition, the nucleic acid sequence may comprise a nucleotide sequence which results from the addition, deletion or substitution of at least one nucleotide to the 5'-end and/or the 3'-end of the nucleic acid formula shown in SEQ ID NO: 1 or a derivative thereof. Any nucleotide or polynucleotide may be used in this regard, provided that its addition, deletion or substitution does not alter the amino acid sequence of SEQ ID NO:2 which is encoded by the nucleotide sequence. For example, the present invention is intended to include any nucleic acid sequence resulting from the addition of ATG as an initiation codon at the 5'-end of the inventive nucleic acid sequence or its derivative, or from the addition of TTA, TAG or TGA as a termination codon at the 3'-end of the inventive nucleotide sequence or its derivative. Moreover, the nucleic acid molecule of the present invention may, as necessary, have restriction endonuclease recognition sites added to its 5'-end and/or 3'-end.

Such functional alterations of a given nucleic acid sequence afford an opportunity to promote secretion and/or processing of heterologous proteins encoded by foreign nucleic acid sequences fused thereto. All variations of the nucleotide sequence of the ERK-5 genes and fragments thereof permitted by the genetic code are, therefore, included in this invention.

Further, it is possible to delete codons or to substitute one or more codons by codons other than degenerate codons to produce a structurally modified polypeptide, but one which has substantially the same utility or activity of the polypeptide produced by the unmodified nucleic acid molecule. As recognized in the art, the two polypeptides are functionally equivalent, as are the two nucleic acid molecules which give rise to their production, even though the differences between the nucleic acid molecules are not related to degeneracy of the genetic code.

A. Isolation of Nucleic Acid.

In one aspect of the present invention, isolated nucleic acid molecules coding for polypeptides having amino acid sequences corresponding to ERK-5 are provided.

- 5 In particular, the nucleic acid molecule may be isolated from a biological sample containing RNA or DNA.

The nucleic acid molecule may be isolated from a biological sample containing RNA using the techniques of cDNA cloning and subtractive hybridization as previously
10 described (Birkenbach et al., J. of Virology 63:9:4079-4084). The nucleic acid molecule may also be isolated from a cDNA library using a homologous probe.

The nucleic acid molecule may be isolated from a biological sample containing genomic DNA or from a genomic
15 library using techniques well known in the art. Suitable biological samples include, but are not limited to, blood, semen and tissue. The method of obtaining the biological sample will vary depending upon the nature of the sample.

One skilled in the art will realize that the human
20 genome may be subject to slight allelic variations between individuals. Therefore, the isolated nucleic acid molecule is also intended to include allelic variations, so long as the sequence is a functional derivation of the ERK-5 gene.

25 One skilled in the art will realize that organisms other than humans may also contain ERK-5 genes (for example, eukaryotes; more specifically, mammals, birds, fish, and plants; more specifically, gorillas, rhesus monkeys, and chimpanzees). The invention is intended to
30 include, but not be limited to, ERK-5 nucleic acid molecules isolated from the above-described organisms.

B. Synthesis of Nucleic Acid.

Isolated nucleic acid molecules of the present invention are also meant to include those chemically
35 synthesized. For example, a nucleic acid molecule with the nucleotide sequence which codes for the expression

product of an ERK-5 gene may be designed and, if necessary, divided into appropriate smaller fragments. Then an oligomer which corresponds to the nucleic acid molecule, or to each of the divided fragments, may be synthesized. Such synthetic oligonucleotides may be prepared, for example, by the triester method of Matteucci et al., J. Am. Chem. Soc. 103:3185-3191 (1981) or by using an automated DNA synthesizer.

An oligonucleotide may be derived synthetically or by cloning. If necessary, the 5'-ends of the oligomers may be phosphorylated using T4 polynucleotide kinase. Kinasing of single strands prior to annealing or for labeling may be achieved using an excess of the enzyme. If kinasing is for the labeling of probe, the ATP may contain high specific activity radioisotopes. Then, the DNA oligomer may be subjected to annealing and ligation with T4 ligase or the like.

II. Substantially Pure ERK-5 Polypeptides.

In another embodiment, the present invention relates to a substantially pure polypeptide having an amino acid sequence corresponding to ERK-5, or at least 9 contiguous amino acids thereof (preferably, at least 10, 15, 20, or 30 contiguous amino acids thereof). In a preferred embodiment, the polypeptide has the amino acid sequence set forth in SEQ ID NO:2, or mutant or species variation thereof, or at least 9 contiguous amino acids thereof (preferably, at least 10, 15, 20, or 30 contiguous amino acids thereof).

A variety of methodologies known in the art can be utilized to obtain the peptide of the present invention. In one embodiment, the peptide is purified from tissues or cells which naturally produce the peptide. Alternatively, the above-described isolated nucleic acid fragments could be used to express the ERK-5 protein in any organism. The samples of the present invention include cells, protein extracts or membrane extracts of cells, or

biological fluids. The sample will vary based on the assay format, the detection method and the nature of the tissues, cells or extracts used as the sample.

Any eukaryotic organism can be used as a source for the peptide of the invention, as long as the source organism naturally contains such a peptide. As used herein, "source organism" refers to the original organism from which the amino acid sequence of the subunit is derived, regardless of the organism the subunit is expressed in and ultimately isolated from.

One skilled in the art can readily follow known methods for isolating proteins in order to obtain the peptide free of natural contaminants. These include, but are not limited to: size-exclusion chromatography, HPLC, ion-exchange chromatography, and immuno-affinity chromatography.

III. A Nucleic Acid Probe for the Detection of ERK-5.

In another embodiment, the present invention relates to a nucleic acid probe for the detection of the presence of ERK-5 in a sample comprising the above-described nucleic acid molecules or at least 27 contiguous nucleotides thereof (preferably at least 30, 35, 40, or 50 thereof). In another preferred embodiment, the nucleic acid probe has the nucleic acid sequence set forth in SEQ ID NO: 1 or at least 27 contiguous nucleotides thereof (preferably at least 30, 35, 40, or 50 thereof). In another preferred embodiment, the nucleic acid probe encodes the amino acid sequence set forth in SEQ ID NO: 2 or at least 9 contiguous amino acids thereof.

The nucleic acid probe may be used to probe an appropriate chromosomal or cDNA library by usual hybridization methods to obtain another nucleic acid molecule of the present invention. A chromosomal DNA or cDNA library may be prepared from appropriate cells according to recognized methods in the art (cf. Molecular Cloning: A Laboratory Manual, second edition, edited by

Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989).

In the alternative, chemical synthesis is carried out in order to obtain nucleic acid probes having nucleotide
5 sequences which correspond to N-terminal and C-terminal portions of the amino acid sequence of the polypeptide of interest. Thus, the synthesized nucleic acid probes may be used as primers in a polymerase chain reaction (PCR) carried out in accordance with recognized PCR techniques,
10 essentially according to PCR Protocols, A Guide to Methods and Applications, edited by Michael et al., Academic Press, 1990, utilizing the appropriate chromosomal or cDNA library to obtain the fragment of the present invention.

One skilled in the art can readily design such probes
15 based on the sequence disclosed herein using methods of computer alignment and sequence analysis known in the art (cf. Molecular Cloning: A Laboratory Manual, second edition, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989).

20 The hybridization probes of the present invention can be labeled by standard labeling techniques such as with a radiolabel, enzyme label, fluorescent label, biotin-avidin label, chemiluminescence, and the like. After hybridization, the probes may be visualized using known
25 methods.

The nucleic acid probes of the present invention include RNA, as well as DNA probes, such probes being generated using techniques known in the art.

In one embodiment of the above described method, a
30 nucleic acid probe is immobilized on a solid support. Examples of such solid supports include, but are not limited to, plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, and acrylic resins, such as polyacrylamide and latex beads. Techniques
35 for coupling nucleic acid probes to such solid supports are well known in the art.

The test samples suitable for nucleic acid probing methods of the present invention include, for example, cells or nucleic acid extracts of cells, or biological fluids. The sample used in the above-described methods will vary based on the assay format, the detection method and the nature of the tissues, cells or extracts to be assayed. Methods for preparing nucleic acid extracts of cells are well known in the art and can be readily adapted in order to obtain a sample which is compatible with the method utilized.

IV. A Method of Detecting The Presence of ERK-5 in a Sample.

In another embodiment, the present invention relates to a method of detecting the presence of ERK-5 in a sample comprising a) contacting said sample with the above-described nucleic acid probe, under conditions such that hybridization occurs, and b) detecting the presence of said probe bound to said nucleic acid molecule. One skilled in the art would select the nucleic acid probe according to techniques known in the art as described above. Samples to be tested include but should not be limited to RNA samples of human tissue.

ERK-5 has been found to be predominantly expressed in muscle. Accordingly, ERK-5 probes may be used to detect the presence of ERK-5 RNA from muscle in a sample. Further, altered expression levels of ERK-5 RNA in an individual, as compared to normal levels, may indicate the presence of muscular disease or diabetes mellitus. The ERK-5 probes may further be used to assay cellular factor activity in general and specifically in muscle tissue.

V. A Kit for Detecting the Presence of ERK-5 in a Sample.

In another embodiment, the present invention relates to a kit for detecting the presence of ERK-5 in a sample comprising at least one container means having disposed

therein the above-described nucleic acid probe. In a preferred embodiment, the kit further comprises other containers comprising one or more of the following: wash reagents and reagents capable of detecting the presence of bound nucleic acid probe. Examples of detection reagents include, but are not limited to radiolabelled probes, enzymatic labeled probes (horse radish peroxidase, alkaline phosphatase), and affinity labeled probes (biotin, avidin, or streptavidin).

10 In detail, a compartmentalized kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allow the efficient transfer of reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the probe or primers used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, and the like), and containers which contain the reagents used to detect the hybridized probe, bound antibody, amplified product, or the like.

One skilled in the art will readily recognize that the nucleic acid probes described in the present invention can readily be incorporated into one of the established kit formats which are well known in the art.

30 VI. DNA Constructs Comprising a ERK-5 Nucleic Acid Molecule and Cells Containing These Constructs.

In another embodiment, the present invention relates to a recombinant DNA molecule comprising, 5' to 3', a promoter effective to initiate transcription in a host cell and the above-described nucleic acid molecules. In another embodiment, the present invention relates to a

recombinant DNA molecule comprising a vector and an above-described nucleic acid molecules.

In another embodiment, the present invention relates to a nucleic acid molecule comprising a transcriptional
5 region functional in a cell, a sequence complimentary to an RNA sequence encoding an amino acid sequence corresponding to the above-described polypeptide, and a transcriptional termination region functional in said cell.

10 Preferably, the above-described molecules are isolated and/or purified DNA molecules.

In another embodiment, the present invention relates to a cell or organism that contains an above-described nucleic acid molecule.

15 In another embodiment, the peptide is purified from cells which have been altered to express the peptide.

As used herein, a cell is said to be "altered to express a desired peptide" when the cell, through genetic manipulation, is made to produce a protein which it
20 normally does not produce or which the cell normally produces at lower levels. One skilled in the art can readily adapt procedures for introducing and expressing either genomic, cDNA, or synthetic sequences into either eukaryotic or prokaryotic cells.

25 A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory information and such sequences are "operably linked" to nucleotide sequences which encode
30 the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene sequence expression. The precise nature of the regulatory regions needed for gene sequence expression
35 may vary from organism to organism, but shall in general include a promoter region which, in prokaryotes, contains both the promoter (which directs the initiation of RNA

transcription) as well as the DNA sequences which, when transcribed into RNA, will signal synthesis initiation. Such regions will normally include those 5'-non-coding sequences involved with initiation of transcription and translation, such as the TATA box, capping sequence, CAAT sequence, and the like.

If desired, the non-coding region 3' to the sequence encoding an ERK-5 gene may be obtained by the above-described methods. This region may be retained for its transcriptional termination regulatory sequences, such as termination and polyadenylation. Thus, by retaining the 3'-region naturally contiguous to the DNA sequence encoding an ERK-5 gene, the transcriptional termination signals may be provided. Where the transcriptional termination signals are not satisfactorily functional in the expression host cell, then a 3' region functional in the host cell may be substituted.

Two DNA sequences (such as a promoter region sequence and an ERK-5 sequence) are said to be operably linked if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region sequence to direct the transcription of an ERK-5 gene sequence, or (3) interfere with the ability of the an ERK-5 gene sequence to be transcribed by the promoter region sequence. Thus, a promoter region would be operably linked to a DNA sequence if the promoter were capable of effecting transcription of that DNA sequence.

Thus, to express an ERK-5 gene, transcriptional and translational signals recognized by an appropriate host are necessary.

The present invention encompasses the expression of the ERK-5 gene (or a functional derivative thereof) in either prokaryotic or eukaryotic cells. Prokaryotic hosts are, generally, very efficient and convenient for the production of recombinant proteins and are, therefore, one type of preferred expression system for the ERK-5 gene.

Prokaryotes most frequently are represented by various strains of *E. coli*. However, other microbial strains may also be used, including other bacterial strains.

5 In prokaryotic systems, plasmid vectors that contain replication sites and control sequences derived from a species compatible with the host may be used. Examples of suitable plasmid vectors may include pBR322, pUC118, pUC119 and the like; suitable phage or bacteriophage
10 vectors may include γ gt10, γ gt11 and the like; and suitable virus vectors may include pMAM-neo, pKRC and the like. Preferably, the selected vector of the present invention has the capacity to replicate in the selected host cell.

15 Recognized prokaryotic hosts include bacteria such as *E. coli*, *Bacillus*, *Streptomyces*, *Pseudomonas*, *Salmonella*, *Serratia*, and the like. However, under such conditions, the peptide will not be glycosylated. The prokaryotic host must be compatible with the replicon and control sequences
20 in the expression plasmid.

To express ERK-5 (or a functional derivative thereof) in a prokaryotic cell, it is necessary to operably link the ERK-5 sequence to a functional prokaryotic promoter. Such promoters may be either constitutive or, more
25 preferably, regulatable (i.e., inducible or derepressible). Examples of constitutive promoters include the *int* promoter of bacteriophage λ , the *bla* promoter of the β -lactamase gene sequence of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase
30 gene sequence of pPR325, and the like. Examples of inducible prokaryotic promoters include the major right and left promoters of bacteriophage λ (P_L and P_R), the *trp*, *recA*, *lacZ*, *lacI*, and *gal* promoters of *E. coli*, the α -amylase (Ulmanen et al., *J. Bacteriol.* 162:176-182(1985))
35 and the ζ -28-specific promoters of *B. subtilis* (Gilman et al., *Gene* sequence 32:11-20(1984)), the promoters of the bacteriophages of *Bacillus* (Gryczan, In: *The Molecular*

Biology of the Bacilli, Academic Press, Inc., NY (1982)), and Streptomyces promoters (Ward et al., Mol. Gen. Genet. 203:468-478 (1986)).

Prokaryotic promoters are reviewed by Glick (J. Ind. Microbiol. 1:277-282 (1987)); Cenatiempo (Biochimie 68:505-516 (1986)); and Gottesman (Ann. Rev. Genet. 18:415-442 (1984)).

Proper expression in a prokaryotic cell also requires the presence of a ribosome binding site upstream of the gene sequence-encoding sequence. Such ribosome binding sites are disclosed, for example, by Gold et al. (Ann. Rev. Microbiol. 35:365-404 (1981)).

The selection of control sequences, expression vectors, transformation methods, and the like, are dependent on the type of host cell used to express the gene. As used herein, "cell", "cell line", and "cell culture" may be used interchangeably and all such designations include progeny. Thus, the words "transformants" or "transformed cells" include the primary subject cell and cultures derived therefrom, without regard to the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. However, as defined, mutant progeny have the same functionality as that of the originally transformed cell.

Host cells which may be used in the expression systems of the present invention are not strictly limited, provided that they are suitable for use in the expression of the ERK-5 peptide of interest. Suitable hosts may often include eukaryotic cells.

Preferred eukaryotic hosts include, for example, yeast, fungi, insect cells, mammalian cells either in vivo, or in tissue culture. Mammalian cells which may be useful as hosts include HeLa cells, cells of fibroblast origin such as VERO or CHO-K1, or cells of lymphoid origin and their derivatives. Preferred mammalian host cells include SP2/0 and J558L, as well as neuroblastoma cell

lines such as IMR 332 which may provide better capacities for correct post-translational processing.

In addition, plant cells are also available as hosts, and control sequences compatible with plant cells are
5 available, such as the cauliflower mosaic virus 35S and 19S, and nopaline synthase promoter and polyadenylation signal sequences.

Another preferred host is an insect cell, for example the *Drosophila* larvae. Using insect cells as hosts, the
10 *Drosophila* alcohol dehydrogenase promoter can be used. Rubin, Science 240:1453-1459(1988). Alternatively, baculovirus vectors can be engineered to express large amounts of ERK-5 in insects cells (Jasny, Science 238:1653 (1987); Miller et al., In: Genetic Engineering (1986),
15 Setlow, J.K., et al., eds., Plenum, Vol. 8, pp. 277-297).

Any of a series of yeast gene sequence expression systems can be utilized which incorporate promoter and termination elements from the actively expressed gene sequences coding for glycolytic enzymes are produced in
20 large quantities when yeast are grown in mediums rich in glucose. Known glycolytic gene sequences can also provide very efficient transcriptional control signals.

Yeast provides substantial advantages in that it can also carry out post-translational peptide modifications.
25 A number of recombinant DNA strategies exist which utilize strong promoter sequences and high copy number of plasmids which can be utilized for production of the desired proteins in yeast. Yeast recognizes leader sequences on cloned mammalian gene sequence products and secretes
30 peptides bearing leader sequences (i.e., pre-peptides). For a mammalian host, several possible vector systems are available for the expression of ERK-5.

A wide variety of transcriptional and translational regulatory sequences may be employed, depending upon the
35 nature of the host. The transcriptional and translational regulatory signals may be derived from viral sources, such as adenovirus, bovine papilloma virus, cytomegalovirus,

simian virus, or the like, where the regulatory signals are associated with a particular gene sequence which has a high level of expression. Alternatively, promoters from mammalian expression products, such as actin, collagen, myosin, and the like, may be employed. Transcriptional initiation regulatory signals may be selected which allow for repression or activation, so that expression of the gene sequences can be modulated. Of interest are regulatory signals which are temperature-sensitive so that by varying the temperature, expression can be repressed or initiated, or are subject to chemical (such as metabolite) regulation.

As discussed above, expression of ERK-5 in eukaryotic hosts requires the use of eukaryotic regulatory regions. Such regions will, in general, include a promoter region sufficient to direct the initiation of RNA synthesis. Preferred eukaryotic promoters include, for example, the promoter of the mouse metallothionein I gene sequence (Hamer et al., J. Mol. Appl. Gen. 1:273-288(1982)); the TK promoter of Herpes virus (McKnight, Cell 31:355-365(1982)); the SV40 early promoter (Benoist et al., Nature (London) 290:304-310(1981)); the yeast gal4 gene sequence promoter (Johnston et al., Proc. Natl. Acad. Sci. (USA) 79:6971-6975(1982); Silver et al., Proc. Natl. Acad. Sci. (USA) 81:5951-5955 (1984)).

As is widely known, translation of eukaryotic mRNA is initiated at the codon which encodes the first methionine. For this reason, it is preferable to ensure that the linkage between a eukaryotic promoter and a DNA sequence which encodes ERK-5 (or a functional derivative thereof) does not contain any intervening codons which are capable of encoding a methionine (i.e., AUG). The presence of such codons results either in a formation of a fusion protein (if the AUG codon is in the same reading frame as the ERK-5 coding sequence) or a frame-shift mutation (if the AUG codon is not in the same reading frame as the ERK-5 coding sequence).

An ERK-5 nucleic acid molecule and an operably linked promoter may be introduced into a recipient prokaryotic or eukaryotic cell either as a nonreplicating DNA (or RNA) molecule, which may either be a linear molecule or, more preferably, a closed covalent circular molecule. Since such molecules are incapable of autonomous replication, the expression of the gene may occur through the transient expression of the introduced sequence. Alternatively, permanent expression may occur through the integration of the introduced DNA sequence into the host chromosome.

In one embodiment, a vector is employed which is capable of integrating the desired gene sequences into the host cell chromosome. Cells which have stably integrated the introduced DNA into their chromosomes can be selected by also introducing one or more markers which allow for selection of host cells which contain the expression vector. The marker may provide for prototrophy to an auxotrophic host, biocide resistance, e.g., antibiotics, or heavy metals, such as copper, or the like. The selectable marker gene sequence can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection. Additional elements may also be needed for optimal synthesis of single chain binding protein mRNA. These elements may include splice signals, as well as transcription promoters, enhancers, and termination signals. cDNA expression vectors incorporating such elements include those described by Okayama, Molec. Cell. Biol. 3:280(1983).

In a preferred embodiment, the introduced nucleic acid molecule will be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors may be employed for this purpose. Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which

do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species. Preferred prokaryotic
5 vectors include plasmids such as those capable of replication in *E. coli* (such as, for example, pBR322, ColE1, pSC101, pACYC 184, π VX. Such plasmids are, for example, disclosed by Sambrook (cf. Molecular Cloning: A Laboratory Manual, second edition, edited by Sambrook,
10 Fritsch, & Maniatis, Cold Spring Harbor Laboratory, (1989)). *Bacillus* plasmids include pC194, pC221, pT127, and the like. Such plasmids are disclosed by Gryczan (In: The Molecular Biology of the Bacteria, Academic Press, NY (1982), pp. 307-329). Suitable *Streptomyces* plasmids
15 include p1J101 (Kendall et al., J. Bacteriol. 169:4177-4183 (1987)), and *streptomyces* bacteriophages such as ϕ C31 (Chater et al., In: Sixth International Symposium on Actinomycetales Biology, Akademiai Kiado, Budapest, Hungary (1986), pp. 45-54). *Pseudomonas* plasmids are
20 reviewed by John et al. (Rev. Infect. Dis. 8:693-704 (1986)), and Izaki (Jpn. J. Bacteriol. 33:729-742 (1978)).

Preferred eukaryotic plasmids include, for example, BPV, vaccinia, SV40, 2-micron circle, and the like, or
25 their derivatives. Such plasmids are well known in the art (Botstein et al., Miami Wntr. Symp. 19:265-274 (1982); Broach, In: The Molecular Biology of the Yeast *Saccharomyces*: Life Cycle and Inheritance, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, p. 445-470
30 (1981); Broach, Cell 28:203-204 (1982); Bollon et al., J. Clin. Hematol. Oncol. 10:39-48 (1980); Maniatis, In: Cell Biology: A Comprehensive Treatise, Vol. 3, Gene Sequence Expression, Academic Press, NY, pp. 563-608 (1980).

Once the vector or nucleic acid molecule containing
35 the construct(s) has been prepared for expression, the DNA construct(s) may be introduced into an appropriate host cell by any of a variety of suitable means, i.e.,

transformation, transfection, conjugation, protoplast fusion, electroporation, particle gun technology, calcium phosphate-precipitation, direct microinjection, and the like. After the introduction of the vector, recipient
5 cells are grown in a selective medium, which selects for the growth of vector-containing cells. Expression of the cloned gene molecule(s) results in the production of ERK-5 or fragments thereof. This can take place in the transformed cells as such, or following the induction of
10 these cells to differentiate (for example, by administration of bromodeoxyuracil to neuroblastoma cells or the like).

A variety of incubation conditions can be used to form the peptide of the present invention. The most
15 preferred conditions are those which mimic physiological conditions.

VII. An Antibody Having Binding Affinity to an ERK-5 Polypeptide, or a Binding Fragment Thereof and a Hybridoma Containing the Antibody.

20 In another embodiment, the present invention relates to an antibody having binding affinity to an ERK-5 polypeptide, or a binding fragment thereof. In a preferred embodiment, the polypeptide has the amino acid sequence set forth in SEQ ID NO:2, or mutant or species
25 variation thereof, or at least 9 contiguous amino acids thereof (preferably, at least 10, 15, 20, or 30 contiguous amino acids thereof).

In another preferred embodiment, the present invention relates to an antibody having binding affinity
30 to an ERK-5 polypeptide, or a binding fragment thereof and not to ERK-1, ERK-2, ERK-3, or ERK-4. Such an antibody may be isolated by comparing its binding affinity to ERK-5 with its binding affinity to ERK-1, ERK-2, ERK-3, or ERK-4. Those which bind selectively to ERK-5 would be chosen
35 for use in methods requiring a distinction between ERK-5 and ERK-1, ERK-2, ERK-3, or ERK-4 polypeptides. Such

methods could include, but should not be limited to, the analysis of altered ERK-5 expression in tissue containing ERK-1, ERK-2, ERK-3, or ERK-4.

5 The ERK-5 proteins of the present invention can be used in a variety of procedures and methods, such as for the generation of antibodies, for use in identifying pharmaceutical compositions, and for studying DNA/protein interaction.

10 The ERK-5 peptide of the present invention can be used to produce antibodies or hybridomas. One skilled in the art will recognize that if an antibody is desired, such a peptide would be generated as described herein and used as an immunogen.

15 The antibodies of the present invention include monoclonal and polyclonal antibodies, as well fragments of these antibodies, and humanized forms. Humanized forms of the antibodies of the present invention may be generated using one of the procedures known in the art such as chimerization or CDR grafting.

20 In another embodiment, the present invention relates to a hybridoma which produces the above-described monoclonal antibody, or binding fragment thereof. A hybridoma is an immortalized cell line which is capable of secreting a specific monoclonal antibody.

25 In general, techniques for preparing monoclonal antibodies and hybridomas are well known in the art (Campbell, "Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology," Elsevier Science Publishers, Amsterdam, The Netherlands
30 (1984); St. Groth et al., J. Immunol. Methods 35:1-21(1980)).

Any animal (mouse, rabbit, and the like) which is known to produce antibodies can be immunized with the selected polypeptide. Methods for immunization are well
35 known in the art. Such methods include subcutaneous or intraperitoneal injection of the polypeptide. One skilled in the art will recognize that the amount of polypeptide

used for immunization will vary based on the animal which is immunized, the antigenicity of the polypeptide and the site of injection.

The polypeptide may be modified or administered in an adjuvant in order to increase the peptide antigenicity. Methods of increasing the antigenicity of a polypeptide are well known in the art. Such procedures include coupling the antigen with a heterologous protein (such as globulin or β -galactosidase) or through the inclusion of an adjuvant during immunization.

For monoclonal antibodies, spleen cells from the immunized animals are removed, fused with myeloma cells, such as SP2/0-Ag14 myeloma cells, and allowed to become monoclonal antibody producing hybridoma cells.

Any one of a number of methods well known in the art can be used to identify the hybridoma cell which produces an antibody with the desired characteristics. These include screening the hybridomas with an ELISA assay, western blot analysis, or radioimmunoassay (Lutz et al., Exp. Cell Res. 175:109-124(1988)).

Hybridomas secreting the desired antibodies are cloned and the class and subclass is determined using procedures known in the art (Campbell, Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology, supra (1984)).

For polyclonal antibodies, antibody containing antisera is isolated from the immunized animal and is screened for the presence of antibodies with the desired specificity using one of the above-described procedures.

In another embodiment of the present invention, the above-described antibodies are detectably labeled. Antibodies can be detectably labeled through the use of radioisotopes, affinity labels (such as biotin, avidin, and the like), enzymatic labels (such as horse radish peroxidase, alkaline phosphatase, and the like) fluorescent labels (such as FITC or rhodamine, and the like), paramagnetic atoms, and the like. Procedures for

accomplishing such labeling are well-known in the art, for example, see (Stemberger et al., J. Histochem. Cytochem. 18:315(1970); Bayer et al., Meth. Enzym. 62:308(1979); Engval et al., Immunot. 109:129(1972); Goding, J. Immunol. 5 Meth. 13:215(1976)). The labeled antibodies of the present invention can be used for in vitro, in vivo, and in situ assays to identify cells or tissues which express a specific peptide.

In another embodiment of the present invention the 10 above-described antibodies are immobilized on a solid support. Examples of such solid supports include plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, acrylic resins and such as polyacrylamide and latex beads. Techniques for coupling 15 antibodies to such solid supports are well known in the art (Weir et al., "Handbook of Experimental Immunology" 4th Ed., Blackwell Scientific Publications, Oxford, England, Chapter 10(1986); Jacoby et al., Meth. Enzym. 34 Academic Press, N.Y. (1974)). The immobilized antibodies 20 of the present invention can be used for in vitro, in vivo, and in situ assays as well as in immunochromotography.

Furthermore, one skilled in the art can readily adapt currently available procedures, as well as the techniques, 25 methods and kits disclosed above with regard to antibodies, to generate peptides capable of binding to a specific peptide sequence in order to generate rationally designed antipeptide peptides, for example see Hurby et al., "Application of Synthetic Peptides: Antisense 30 Peptides", In Synthetic Peptides, A User's Guide, W.H. Freeman, NY, pp. 289-307(1992), and Kaspczak et al., Biochemistry 28:9230-8(1989).

Anti-peptide peptides can be generated in one of two fashions. First, the anti-peptide peptides can be 35 generated by replacing the basic amino acid residues found in the ERK-5 peptide sequence with acidic residues, while maintaining hydrophobic and uncharged polar groups. For

example, lysine, arginine, and/or histidine residues are replaced with aspartic acid or glutamic acid and glutamic acid residues are replaced by lysine, arginine or histidine.

5 VIII. A Method of Detecting an ERK-5 Polypeptide in a Sample.

In another embodiment, the present invention relates to a method of detecting an ERK-5 polypeptide in a sample, comprising: a) contacting the sample with an above-
10 described antibody, under conditions such that immunocomplexes form, and b) detecting the presence of said antibody bound to the polypeptide. In detail, the methods comprise incubating a test sample with one or more of the antibodies of the present invention and assaying
15 whether the antibody binds to the test sample. Altered levels of ERK-5 in a sample as compared to normal levels may indicate muscular disease.

Conditions for incubating an antibody with a test sample vary. Incubation conditions depend on the format
20 employed in the assay, the detection methods employed, and the type and nature of the antibody used in the assay. One skilled in the art will recognize that any one of the commonly available immunological assay formats (such as radioimmunoassays, enzyme-linked immunosorbent assays,
25 diffusion based Ouchterlony, or rocket immunofluorescent assays) can readily be adapted to employ the antibodies of the present invention. Examples of such assays can be found in Chard, "An Introduction to Radioimmunoassay and Related Techniques" Elsevier Science Publishers,
30 Amsterdam, The Netherlands (1986); Bullock et al., "Techniques in Immunocytochemistry," Academic Press, Orlando, FL Vol. 1 (1982), Vol. 2 (1983), Vol. 3 (1985); Tijssen, "Practice and Theory of Enzyme Immunoassays: Laboratory Techniques in Biochemistry and Molecular
35 Biology," Elsevier Science Publishers, Amsterdam, The Netherlands (1985).

The immunological assay test samples of the present invention include cells, protein or membrane extracts of cells, or biological fluids such as blood, serum, plasma, or urine. The test sample used in the above-described method will vary based on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can be readily be adapted in order to obtain a sample which is capable with the system utilized.

IX. A Diagnostic Kit Comprising Antibodies to ERK-5.

In another embodiment of the present invention, a kit is provided which contains all the necessary reagents to carry out the previously described methods of detection. The kit may comprise: i) a first container means containing an above-described antibody, and ii) second container means containing a conjugate comprising a binding partner of the antibody and a label. In another preferred embodiment, the kit further comprises one or more other containers comprising one or more of the following: wash reagents and reagents capable of detecting the presence of bound antibodies. Examples of detection reagents include, but are not limited to, labeled secondary antibodies, or in the alternative, if the primary antibody is labeled, the chromophoric, enzymatic, or antibody binding reagents which are capable of reacting with the labeled antibody. The compartmentalized kit may be as described above for nucleic acid probe kits.

One skilled in the art will readily recognize that the antibodies described in the present invention can readily be incorporated into one of the established kit formats which are well known in the art.

X. Isolation of Compounds Which Interact With ERK-5.

In another embodiment, the present invention relates to a method of detecting a compound capable of binding to

ERK-5 or a fragment thereof comprising incubating the compound with ERK-5 or fragment thereof and detecting the presence of the compound bound to ERK-5 or fragment thereof. In a preferred embodiment, the compound is
5 present within a complex mixture, for example, serum, body fluid, or cell extracts.

In another embodiment, the present invention relates to a method of detecting an agonist or antagonist of ERK-5 activity comprising incubating cells that produce ERK-5 in
10 the presence of a compound and detecting changes in the level of ERK-5 activity. The compounds thus identified would produce a change in activity indicative of the presence of the compound. In a preferred embodiment, the compound is present within a complex mixture, for example,
15 serum, body fluid, or cell extracts. Once the compound is identified it can be isolated using techniques well known in the art.

In a further embodiment, the present invention relates to a method of agonizing (stimulating) or
20 antagonizing ERK-5 associated activity in a mammal comprising administering to said mammal an agonist or antagonist to ERK-5 in an amount sufficient to effect said agonism or antagonism. In a preferred embodiment, the present invention relates to a method of treating diabetes
25 mellitus, skeletal muscle diseases, Alzheimer's disease, or peripheral neuropathies in a mammal with an agonist or antagonist of ERK-5 activity comprising administering the agonist or antagonist to a mammal in an amount sufficient to agonize or antagonize ERK-5 associated functions.
30 Further, since ERK-5 is preferentially expressed in skeletal muscle, the agonist or antagonist might be used in normal individuals.

One skilled in the art will appreciate that the amounts to be administered for any particular treatment
35 protocol can readily be determined. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the

like. Generally, the dosage will vary with the age, condition, sex and extent of disease in the patient, counter indications, if any, and other such variables, to be adjusted by the individual physician. Dosage can vary
5 from .001 mg/kg to 50 mg/kg, preferably 0.1 mg/kg to 1.0 mg/kg, of the agonist or antagonist of the invention, in one or more administrations daily, for one or several days. The agonist or antagonist can be administered parenterally by injection or by gradual perfusion over
10 time. They can be administered intravenously, intraperitoneally, intramuscularly, or subcutaneously.

Preparations for parenteral administration include sterile or aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are
15 propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium
20 chloride solution, Ringer's dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also
25 be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like. See, generally, Remington's Pharmaceutical Science, 16th Ed., Mack Eds. (1980).

In another embodiment, the present invention relates
30 to a pharmaceutical composition comprising the above described ERX-5 agonist or antagonist in an amount sufficient to alter ERK-5 associated activity, and a pharmaceutically acceptable diluent, carrier, or excipient. Appropriate concentrations and dosage unit
35 sizes can be readily determined by one skilled in the art as described above (See, for example, Remington's

Pharmaceutical Sciences (16th ed., Osol, A., Ed., Mack, Easton PA (1980) and WO 91/19008).

The present invention is described in further detail in the following non-limiting examples.

5 Example 1

Screening of a Human Skeletal Muscle cDNA
Library with Radiolabelled Oligonucleotides

Total RNA was isolated from human skeletal muscle by the acid guanidinium thiocyanate-phenol-chloroform
10 extraction procedure as described by Puissant et al., BioTechniques 8:148-149(1990). Poly (A+) RNA was isolated on an oligo(dT) column (Avid et al., Proc. Natl. Acad. Sci. USA 69:1408-1412(1972)). A cDNA library was constructed using the methods described by Okayama and
15 Berg, Mol. Cell. Biol. 2:161-170(1982); Okayama and Berg, Mol. Cell. Biol. 3:280-289(1983). The pCDVI-PL vector was used for preparation of the primer fragment (Noma et al., Nature 319:640-646 (1986)). A short synthetic adapter was used as second strand primer as recently described (Boel,
20 E. et al., BioTechniques 11(1):26-28 (July, 1991)). E. coil DH5 α (Gibco BRL, Gaithersburg, MD 20877, USA) was used for transformation according to the protocols by H. Inouue et al., Gene 96:23-28 (1990). After transformation, the bacteria were plated on LB plates containing 50 μ g/ml
25 ampicillin at a density of about 8000 colonies per 15 cm plate. Nitrocellulose replica filters (Schleicher & Schuell, BA85) were screened with standard colony hybridization technique (Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, New York
30 1989). An equimolar mixture of the following three oligonucleotides which were labelled at the 5' end using T4 polynucleotide kinase and [γ - 32 P] ATP (Amersham, Braunschweig) (Sambrook et al., 1989) was used for hybridization.

E10 5' AAG GGT TTT ACC ATG GCA GAG AAA 3' SEQ ID NO:3

Met Ala Glu Lys SEQ ID NO:4

E11 5' TTA ACT TGT CGA CTA CGT CAG CAG 3' SEQ ID NO:5

E13 5' A(CT) AT(GT) TGG (GT)CT (AG) GGC TGC ATC 3'

5 SEQ ID NO:6

The nucleotide sequences of the oligonucleotides correspond to nucleotides (nt) 378-401 (E10, including the first in frame methionine-codon) and nt 2033-2010 (E11, reverse primer, including the first in frame stop codon), respectively, of the rat ERK-3 sequence published by Boulton et al., Cell 65:663-675(1991) with single modifications outside the coding region to introduce new restriction sites (NcoI in E10 and SalI in E11, respectively). E13 was designed as 32 fold degenerate oligonucleotide based on the amino acid sequence of rat ERK1, ERK2 and ERK3 corresponding to nt 1030-1052 of rat erk3 (Boulton et al., Cell 65:663-675 (1991)).

A total of 10 pmoles of the labelled oligonucleotides E10, E11 and E13 in 50 ml hybridization mixture (6xSSC, 5x Denhardt's solution, 0.05% SDS (Current Protocols in Molecular Biology, M. Ausubei et al., eds., John Wiley & Sons, New York (1988)) were added to replica nitrocellulose filters and allowed to hybridize at 42° C for 2 h. The filters were washed in 6xSSC, 0.05% SDS three times 10 min first at room temperature, then at 42°, 46°, 48° and 50° C, respectively.

Two positive clones were identified by autoradiography and isolated following the procedure described in Sambrook et al., 1989. Partial dideoxy sequencing (Sanger et al., Proc. Natl. Acad. Sci. USA 74:5463-5467(1977)) of the two positive clones using oligonucleotide E13 as primer revealed identical nucleotide sequence. The larger clone with an insert of about 1900 bp was then fully sequenced. The first methionine precedes an open reading frame of 1179 bp encoding a protein of 393 amino acids (Figure 1, SEQ ID NO:1 and SEQ ID NO:2) or 43.2 kD molecular weight which

shows 61% similarity (38% identity) to the human ERK1 peptide sequence, 64% similarity to the rat ERK1 and ERK2 (39% and 37% identity, respectively) and 55% similarity to the rat ERK3 (30% identity) (the comparison program uses the algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443-453 (1970)). The new clone was termed human ERK5 (hERK5).

Example 2

Northern Blot Analysis of Human ERK5

10 Total RNA from human tissue was isolated by the acid guanidinium thiocyanate-phenol-chloroform extraction procedure (Puissant and Houdebine, BioTechniques 8:148-149 (1990)). The preparation of poly(A+) RNA was performed as described by Aviv and Leder, Proc. Natl. Acad. Sci. USA
15 69:1408-1412 (1972). Five μ g of poly(A+) RNA per lane were loaded on a 1.2% agarose-2.2 M formaldehyde gel and after separation blotted onto a nitrocellulose filter using standard techniques (Sambrook et al., 1989).

A 32 P labelled 1200 bp Bam HI fragment of hERK5 in 30
20 ml of hybridization solution (5x Denhardt's solution, 5xSSC, 5 μ g/ml salmon sperm DNA, 50 mM Na_2HPO_4 , pH 6.8, 1 mM $\text{NaH}_2\text{PO}_4/\text{Na}_4\text{P}_2\text{O}_4$, 50% formamide) was used for hybridization. The 32 P labelling was done using the Random primed DNA labelling Kit (Bat No. 1004760, Boehringer Mannheim
25 Biochemica) according to the manufacturers instruction. Fifty ng of denatured DNA were labelled with 50 μ Ci 32 PdATP with an average incorporation of 2×10^8 - 1×10^9 cpm/ μ g DNA. After the labelling reaction unincorporated 32 PdATP was removed using a Sephadex G50 column. The washed filter
30 was exposed to an X-ray film. Analysis of the Northern Blot showed that there is a major transcript of hERK5 of about 1.9 kb (Figure 2). Further, it appears that hERK5 is preferentially expressed in skeletal muscle.

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Example 3Production of Polyclonal Antibodies Against hERK5

Antibodies were raised against an E. coli fusion protein composed of the carboxy-terminal part of glutathione S-transferase and the last 264 amino acids of hERK5 protein. The vector encoding this construct was generated by cloning a 1200 bp BamHI-fragment of hERK5 into the pGEX3X plasmid (Pharmacia, Uppsala) which upstream of the multiple cloning site carries the cDNA for about 250 amino acids (27.5 kD) of glutathione S-transferase under the control of the lac promoter. The construct was transformed in E. coli 298F' cells (R. du Bridge, Genentech, San Francisco). After induction with IPTG (1 mM final concentration) the expressed soluble fusion protein was purified on Glutathione-Sepharose 4B (Pharmacia, Uppsala) according to the manufacturer's instruction. The apparent molecular weight in SDS-PAGE is 56 kD (Figure 3). The purified fusion protein (100 µg) was subcutaneously injected into a female rabbit using Freund's adjuvant as described in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor, New York (1988). After the second boost, antiserum was collected and used in Western blotting.

For Western blotting total lysate of E. coli clones expressing either the pGEX encoded GST protein portion or the hERK-5-GST fusion protein before and after induction with IPTG was separated on SDS-PAGE. The bacteria were harvested by centrifugation and resuspended in Laemmli sample buffer (4% SDS, 125 mM Tris pH 6.8, 10% β-mercapto ethanol, 10% glycerol, 0.02% bromphenol blue) to a concentration of 2×10^7 cells/µl of sample buffer. After boiling for 5 min, 10 µl of this SDS lysate were applied to SDS-PAGE (10% polyacrylamide), transferred to nitrocellulose using standard techniques (Sambrook et al., (1989), 200 mA, 40 min), blocked with PBS containing 2% nonfat dry milk, 0.02% Tween20, washed (PBS 0.02% Tween 20, 0.2% gelatine) and detected with the polyclonal

antiserum raised against the hERK-GST fusion protein in comparison to preimmune serum (both sera diluted 1:5000 in PBS containing 0.05% Tween 20 and 0.2% gelatine). The second antibody (horseradish peroxidase coupled goat anti-rabbit IgG (BioRad, Munchen)) was diluted 1:20000 fold in PBS 0.02% Tween 20, 0.2% gelatine. Peroxidase reaction was performed using the ECL Kit (Amersham, Braunschweig). The antiserum strongly recognizes a band at 56 kD corresponding to the molecular weight of the hERK-GST fusion protein which is not detected by the preimmune serum. There is no cross reactivity of the anti-hEPK-5-GST antiserum with the recombinant GST protein portion (Figure 4).

Example 4

15 Expression of hERK5 in Eukaryotic Cells

The herk 5 cDNA was cloned in the eukaryotic expression vector pcDNAI (Invitrogen, San Diego) and hERK-5 was transiently expressed in human embryonal kidney fibroblasts (293 cells:ATCC CRL 1573). The 293 cells were grown in DMEM with 4.5 mg/ml glucose and 10% FCS. 5x10⁴ cells per 3.5 cm dish were transfected with 10 µg DNA using the calcium-phosphate precipitation method described by Chen and Okayama (Mol. Cell. Biol. 7:2745-2752(1987)). After 16 h at 35°C and 3% CO₂ the medium was changed. The cells were transferred to 37°C and 5% CO₂ for additional 16 h, washed off the cultured dish, collected by centrifugation, and resuspended in Laemmli sample buffer (composition see Example 3, 40 µl per 3.5 cm dish).

The Western blot of 293 cell SDS-lysate after pcDNAI-herk5 transfection shows major bands at 44 kD (which corresponds to the predicted molecule weight of hERK5) and 46 kD, probably representing a different phosphorylation state of hERK5 protein (Figure 5). There is no cross reactivity with proteins expressed in mock transfected 293 cells.

Example 5Generation of Stable Cell Lines Expressing hERK5

NIH3T3 cells, immortalized mouse fibroblasts (Jainchill et al., J. Virol. 4:549-553 (1969)) were grown in DMEM with 4.5 mg/ml glucose and 10% FCS to subconfluency and transfected with 20 μ g/ 1×10^7 cells of a cvn-construct containing the complete hERK-5 cDNA. The cvn vector carries the SV40 early promoter, HBV poly A signal as well as a neomycin resistance gene which allows selection of transfected cells on G418 resistance, and the gene for the DHFR which can be used to increase the expression of the integrated cDNA by addition of methotrexate at concentrations of 100-1000 nM to the culture medium (Rosenthal et al., Cell 46:155-169 (1986)). Transfection was performed as described in Example 4. After 16 h at 35°C and 3% CO₂, the medium was changed and the cells were grown at 37°C, 5% CO₂ for additional 24 h with one medium change after 8 h. The cells were then split to different dilutions and grown in 1mg/ml G418 containing medium until cell colonies appeared which were isolated and selected on methotrexate growth.

The expression of hERK5 was tested in the Western blot of total cell lysate using the antibody raised against the hERK-5-GST fusion protein as described in Example 3. The blot shows a double band at 44/46 kD in three of four cell clones tested corresponding to the stably expressed hERK5 protein whose expression is increased when the cells are grown in 200 and 500 mM methotrexate, respectively (Figure 6).

30

* * * * *

All publications mentioned hereinabove are hereby incorporated in their entirety by reference.

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be appreciated by one skilled in the art from a

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reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention and appended claims.

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SEQUENCE LISTING

- (1) GENERAL INFORMATION:
- (i) APPLICANT: Lechner, Cornelia
Moller, Niels P.H.
5 Ullrich, Axel
- (ii) TITLE OF INVENTION: Extracellular Regulated
Kinase, Sequences, and Methods of Production
and Use
- (iii) NUMBER OF SEQUENCES: 6
- 10 (iv) CORRESPONDENCE ADDRESS:
- (A) ADDRESSEE: Sterne, Kessler, Goldstein &
Fox
- (B) STREET: 1225 Connecticut Avenue NW
- (C) CITY: Washington
- 15 (D) STATE: D.C.
- (E) COUNTRY: U.S.A.
- (F) ZIP: 20036
- (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Floppy disk
- 20 (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version
#1.25
- (vi) CURRENT APPLICATION DATA:
- 25 (A) APPLICATION NUMBER: US (to be assigned)
- (B) FILING DATE: Herewith
- (C) CLASSIFICATION:
- (ix) TELECOMMUNICATION INFORMATION:
- (A) TELEPHONE: (202) 466-0800
- 30 (B) TELEFAX: (202) 833-8716
- (2) INFORMATION FOR SEQ ID NO: 1:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1260 base pairs
- (B) TYPE: nucleic acid
- 35 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ix) FEATURE:

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(A) NAME/KEY: CDS

(B) LOCATION: 34..1215

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

5	GGCTCTGCGG	GGTGGGCAGC	TCCCGGGCCT	GCC	ATG	AGC	TCT	CCG	CCG	CCC	GGN	54					
					Met	Ser	Ser	Pro	Pro	Pro	Gly						
					1					5							
	GGC	AGT	GGC	TTT	TAC	CGC	CAG	GAG	GTG	ACC	AAG	ACG	GCC	TGG	GAG	GTG	102
	Gly	Ser	Gly	Phe	Tyr	Arg	Gln	Glu	Val	Thr	Lys	Thr	Ala	Trp	Glu	Val	
			10					15					20				
10	CGC	GCC	GTG	TAC	CGG	GAC	CTG	CAG	CCC	GTG	GGC	TCG	GGC	GCC	TAC	GGC	150
	Arg	Ala	Val	Tyr	Arg	Asp	Leu	Gln	Pro	Val	Gly	Ser	Gly	Ala	Tyr	Gly	
		25					30				35						
	CGC	GTG	TGC	TCG	GCC	GTG	GAC	GGC	CGC	ACC	GGC	GCT	AAG	GTT	GCC	ATC	198
	Ala	Val	Cys	Ser	Ala	Val	Asp	Gly	Arg	Thr	Gly	Ala	Lys	Val	Ala	Ile	
15		40				45					50						
	AAG	AAG	CTG	TAT	CGG	CCC	TTC	CAG	TCC	GAG	CTG	TTC	GCC	AAG	CTC	GCC	246
	Lys	Lys	Leu	Tyr	Arg	Pro	Phe	Gln	Ser	Glu	Leu	Phe	Ala	Lys	Leu	Ala	
					60					65					70		
	TAC	CGC	GAG	CTG	CGC	CTG	CTC	AAG	CAC	ATG	CGC	CAC	GAG	AAC	GTG	ATC	294
20	Tyr	Arg	Glu	Leu	Arg	Leu	Leu	Lys	His	Met	Arg	His	Glu	Asn	Val	Ile	
				75				80						85			
	GGG	CTG	CTG	GAC	GTA	TTC	ACT	CCT	GAT	GAG	ACC	CTG	GAT	GAC	TTC	ACG	342
	Gly	Leu	Leu	Asp	Val	Phe	Thr	Pro	Asp	Glu	Thr	Leu	Asp	Asp	Phe	Thr	
			90					95					100				
25	GAC	TTT	TAC	CTG	GTG	ATG	CCG	TTC	ATG	GGC	ACC	GAC	CTG	GGC	AAG	CTC	390
	Asp	Phe	Tyr	Leu	Val	Met	Pro	Phe	Met	Gly	Thr	Asp	Leu	Gly	Lys	Leu	
		105					110					115					
	ATG	AAA	CAT	GAG	AAG	CTA	GGC	GAG	GAC	CGG	ATC	CAG	TTC	CTC	GTG	TAC	438
	Met	Lys	His	Glu	Lys	Leu	Gly	Glu	Asp	Arg	Ile	Gln	Phe	Leu	Val	Tyr	
30		120				125					130					135	
	CAG	ATG	ATG	AAG	GGG	CTG	AGG	TAT	ATC	CAC	GCT	GCC	GGC	ATC	ATC	CAC	486
	Gln	Met	Met	Lys	Gly	Leu	Arg	Tyr	Ile	His	Ala	Ala	Gly	Ile	Ile	His	
					140					145					150		
35	AGA	GAC	CTG	AAG	CCC	GGC	AAC	CTG	GCT	GTG	AAC	GAA	GAC	TGT	GAG	CTG	534
	Arg	Asp	leu	Lys	Pro	Gly	Asn	Leu	Ala	Val	Asn	Glu	Asp	Cys	Glu	Leu	
				155					160					165			
	AAG	ATC	CTG	GAC	TTC	GGC	CTG	GCC	AGG	CAG	GCA	GAC	AGT	GAG	ATG	ACT	582
	Lys	Ile	Leu	Asp	Phe	Gly	Leu	Ala	Arg	Gln	Ala	Asp	Ser	Glu	Met	Thr	
			170					175					180				
40	GGG	TAC	GTG	GTG	ACC	CGG	TGG	TAC	CGG	GCT	CCC	GAG	GTC	ATC	TTG	AAT	630
	Gly	Tyr	Val	Val	Thr	Arg	Trp	Tyr	Arg	Ala	Pro	Glu	Val	Ile	Leu	Asn	
		185					190					195					
	TGG	ATC	GCG	TAC	ACG	CAG	ACG	GTG	GAC	ATC	TGG	TCT	GTG	GGC	TGC	ATC	678
	Trp	Ile	Ala	Tyr	Thr	Gln	Thr	Val	Asp	Ile	Trp	Ser	Val	Gly	Cys	Ile	
45		200				205					210				215		
	ATG	GCG	GAG	ATG	ATC	ACA	GGC	AAG	ACG	CTG	TTC	AAG	GGC	AGC	GAC	CAC	726
	Met	Ala	Glu	Met	Ile	Thr	Gly	Lys	Thr	Leu	Phe	Lys	Gly	Ser	Asp	His	
					220					225					230		

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CTG GAC CAG CTG AAG GAG ATC ATG AAG GTG ACG GGG ACG CCT CCG GCT 774
 Leu Asp Gln Leu Lys Glu Ile Met Lys Val Thr Gly Thr Pro Pro Ala
 235 240 245

5 GAG TTT GTG CAG CGG CTG CAG AGC GAT GAG GCC AAG AAC TAC ATG AAG 822
 Glu Phe Val Gln Arg Leu Gln Ser Asp Glu Ala Lys Asn Tyr Met Lys
 250 255 260

GGC CTC CCC GAA TTG GAG AAG AAG GAT TTT GCC TCT ATC CTG ACC AAT 870
 Gly Leu Pro Glu Leu Glu Lys Lys Asp Phe Ala Ser Ile Leu Thr Asn
 265 270 275

10 GCA AGC CCT CTG GCT GTG AAC CTC CTG GAG AAG ATG CTG GTG CTG GAC 918
 Ala Ser Pro Leu Ala Val Asn Leu Leu Glu Lys Met Leu Val Leu Asp
 280 285 290 295

GCG GAC ATC AGG TTG ACT GCA GGC GAG TTT CTT TCC CAT CCC TAC TTC 966
 Ala Asp Ile Arg Leu Thr Ala Gly Glu Phe Leu Ser His Pro Tyr Phe
 300 305 310

15 GAG TCC CTG CAC GAC ACG GAA GAT GAG CCC CAG GTC CAG AAG TAT GAT 1014
 Glu Ser Leu His Asp Thr Glu Asp Glu Pro Gln Val Gln Lys Tyr Asp
 315 320 325

GAC TCC TTT GAC TAC TTT GAC CGC ACA CTG GAT GAA TGG AAG CCG TGT 1062
 Asp Ser Phe Asp Tyr Phe Asp Arg Thr Leu Asp Glu Trp Lys Pro Cys
 330 335 340

TAC TTA CAA AGA GGT GCT CAG CTT CAA GCC TCC CCG GCA GCT GGG GGC 1110
 Tyr Leu Gln Arg Gly Ala Gln Leu Gln Ala Ser Pro Ala Ala Gly Gly
 345 350 355

25 CAG GGT CTC CAA GGA GAC GCC TCT GTG AAG ATC TCT GGG CTC CGG GGT 1158
 Gln Gly Leu Gln Gly Asp Ala Ser Val Lys Ile Ser Gly Leu Arg Gly
 360 365 370 375

GGC AGT GAG GAC CAC CTT CAC CTT CCA CCT GAG AGG GGA CTC TCG TTG 1206
 Gly Ser Glu Asp His Leu His Leu Pro Pro Glu Arg Gly Leu Ser Leu
 380 385 390

30 CCA CCT TGACCTTGGC TGGGGCTTGC ATCCCAAGGC ATCCATCAGA GCAGACGC 1260
 Pro Pro

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 35 (A) LENGTH: 393 amino acids
 (B) TYPE: amino acid
 (C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) SEQUENCE DESCRIPTION: SEQ ID NO:2:

40 Met Ser Ser Pro Pro Pro Gly Gly Ser Gly Phe Tyr Arg Gln Glu Val
 1 5 10 15

Thr Lys Thr Ala Trp Glu Val Arg Ala Val Tyr Arg Asp Leu Gln Pro
 20 25 30

45 Val Gly Ser Gly Ala Tyr Gly Ala Val Cys Ser Ala Val Asp Gly Arg
 35 40 45

Thr Gly Ala Lys Val Ala Ile Lys Lys Leu Tyr Arg Pro Phe Gly Ser
 50 55 60

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	Glu	Leu	Phe	Ala	Lys	Leu	Ala	Tyr	Arg	Glu	Leu	Arg	Leu	Leu	Lys	His
	65					70					75				80	
	Met	Arg	His	Glu	Asn	Val	Ile	Gly	Leu	Leu	Asp	Val	Phe	Thr	Pro	Asp
					85				90						95	
5	Glu	Thr	Leu	Asp	Asp	Phe	Thr	Asp	Phe	Tyr	Leu	Val	Met	Pro	Phe	Met
				100					105					110		
	Gly	Thr	Asp	Leu	Gly	Lys	Leu	Met	Lys	His	Glu	Lys	Leu	Gly	Glu	Asp
			115					120					125			
10	Arg	Ile	Gln	Phe	Leu	Val	Tyr	Gln	Met	Met	Lys	Gly	Leu	Arg	Tyr	Ile
		130					135					140				
	His	Ala	Ala	Gly	Ile	Ile	His	Arg	Asp	Leu	Lys	Pro	Gly	Asn	Leu	Ala
	145					150					155					160
	Val	Asn	Glu	Asp	Cys	Glu	Leu	Lys	Ile	Leu	Asp	Phe	Gly	Leu	Ala	Arg
					165					170					175	
15	Gln	Ala	Asp	Ser	Glu	Met	Thr	Gly	Tyr	Val	Val	Thr	Arg	Trp	Tyr	Arg
				180					185					190		
	Ala	Pro	Glu	Val	Ile	Leu	Asn	Trp	Ile	Ala	Tyr	Thr	Gln	Thr	Val	Asp
			195					200					205			
20	Ile	Trp	Ser	Val	Gly	Cys	Ile	Met	Ala	Glu	Met	Ile	Thr	Gly	Lys	Thr
		210					215					220				
	Leu	Phe	Lys	Gly	Ser	Asp	His	Leu	Asp	Gln	Leu	Lys	Glu	Ile	Met	Lys
	225					230					235					240
	Val	Thr	Gly	Thr	Pro	Pro	Ala	Glu	Phe	Val	Gln	Arg	Leu	Gln	Ser	Asp
					245					250					255	
25	Glu	Ala	Lys	Asn	Tyr	Met	Lys	Gly	Leu	Pro	Glu	Leu	Glu	Lys	Lys	Asp
				260					265					270		
	Phe	Ala	Ser	Ile	Leu	Thr	Asn	Ala	Ser	Pro	Leu	Ala	Val	Asn	Leu	Leu
			275					280					285			
30	Glu	Lys	Met	Leu	Val	Leu	Asp	Ala	Asp	Ile	Arg	Leu	Thr	Ala	Gly	Glu
		290					295					300				
	Phe	Leu	Ser	His	Pro	Tyr	Phe	Glu	Ser	Leu	His	Asp	Thr	Glu	Asp	Glu
	305					310					315					320
	Pro	Gln	Val	Gln	Lys	Tyr	Asp	Asp	Ser	Phe	Asp	Tyr	Phe	Asp	Arg	Thr
					325					330					335	
35	Leu	Asp	Glu	Trp	Lys	Pro	Cys	Tyr	Leu	Gln	Arg	Gly	Ala	Gln	Leu	Gln
				340					345					350		
	Ala	Ser	Pro	Ala	Ala	Gly	Gly	Gln	Gly	Leu	Gln	Gly	Asp	Ala	Ser	Val
			355					360					365			
40	Lys	Ile	Ser	Gly	Leu	Arg	Gly	Gly	Ser	Glu	Asp	His	Leu	His	Leu	Pro
		370					375					380				
	Pro	Glu	Arg	Gly	Leu	Ser	Leu	Pro	Pro							
	385					390										

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(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5

(ix) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AAGGGTTTTA CCATGGCAGA GAAA

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

15 Met Ala Glu Lys

1

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TTAACTTGTC GACTACGTCA GCAG

25 (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ACTATGTTGG GTCTGCTAGG GCTGCATC

Claims

1. An isolated nucleic acid molecule coding for a polypeptide comprising an amino acid sequence corresponding to ERK-5, or at least 9 contiguous amino acids thereof.
2. The isolated nucleic acid molecule according to claim 1, wherein the molecule comprises the sequence set forth in SEQ ID NO: 1, or allelic, mutant or species variation thereof.
3. The isolated nucleic acid molecule according to claim 1, wherein the molecule has the sequence set forth in SEQ ID NO:1.
4. The isolated nucleic acid molecule according to claim 1, wherein the molecule encodes the amino acid sequence set forth in SEQ ID NO:2, or mutant or species variation thereof.
5. The isolated nucleic acid molecule according to claim 1, wherein the molecule encodes the amino acid sequence set forth in SEQ ID NO:2.
6. A substantially pure polypeptide comprising an amino acid sequence corresponding to ERK-5, or at least 9 contiguous amino acids thereof.
7. The polypeptide according to claim 6, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO:2, at least 9 contiguous amino acids thereof or mutant or species variation thereof.
8. The polypeptide according to claim 6, wherein the polypeptide has the amino acid sequence set forth in SEQ ID NO:2.

9. A nucleic acid probe for the detection of the presence of ERK-5 in a sample comprising the molecule according to claim 1 or at least 27 contiguous nucleotides thereof.

5 10. The nucleic acid probe according to claim 9, wherein the molecule has the nucleic acid sequence set forth in SEQ ID NO:1, or at least 27 contiguous nucleotides thereof.

10 11. The nucleic acid probe according to claim 9, wherein the probe encodes the amino acid sequence set forth in SEQ ID NO:2, or at least 9 contiguous amino acids thereof.

12. A method of detecting ERK-5 RNA in a sample comprising:

15 a) contacting said sample with the nucleic acid probe according to claim 9, under conditions such that hybridization occurs, and

b) detecting the presence of said probe bound to ERK-5 RNA.

20 13. A kit for detecting the presence of ERK-5 RNA in a sample comprising at least one container means having disposed therein the nucleic acid probe according to claim 9.

25 14. A recombinant nucleic acid molecule comprising, 5' to 3', a promoter effective to initiate transcription in a host cell and the isolated nucleic acid molecule according to claim 1.

30 15. A recombinant nucleic acid molecule comprising a vector and the isolated nucleic acid molecule according to claim 1.

16. A recombinant nucleic acid molecule comprising a transcriptional region functional in a cell, a sequence complimentary to an RNA sequence encoding an amino acid sequence corresponding to the polypeptide of claim 6, and
5 a transcriptional termination region functional in said cell.

17. A cell that contains the recombinant nucleic acid molecule according to any one of claims 14, 15, or 16.

10 18. An organism that contains the recombinant nucleic acid molecule according to any one of claims 14, 15, or 16.

19. An antibody having binding affinity to an ERK-5 polypeptide, or a binding fragment thereof, and not to
15 ERK-1, ERK-2, ERK-3, or ERK-4.

20. The antibody according to claim 19, wherein said polypeptide comprises at least a contiguous amino acids of the amino acid sequence set forth in SEQ ID NO:2, or mutant or species variation thereof.

20 21. The antibody according to claim 19, wherein said antibody is a monoclonal antibody.

22. A method of detecting an ERK-5 polypeptide in a sample, comprising:

a) contacting said sample with an antibody
25 according to claim 19, under conditions such that immunocomplexes form, and

b) detecting the presence of said antibody bound to said polypeptide.

23. A method of measuring the amount of ERK-5 in a
30 sample, comprising:

a) contacting said sample with an antibody according to claim 19, under conditions such that immunocomplexes form, and

b) measuring the amount of immunocomplexes formed
5 between said antibodies and any ERK-5 in said sample.

24. A diagnostic Kit comprising:

i) a first container means containing the antibody according to claim 19, and

ii) second container means containing a
10 conjugate comprising a binding partner of said monoclonal antibody and a label.

25. A hybridoma which produces the monoclonal antibody according to claim 19 or 20.

26. A method of detecting a compound capable of
15 binding to ERK-5 or a fragment thereof comprising incubating the compound with ERK-5 or fragment thereof and detecting the presence of the compound bound to said ERK-5 or fragment thereof.

27. A method of detecting an agonist or antagonist
20 of ERK-5 activity comprising incubating cells that produce ERK-5 in the presence of a compound and detecting changes in the level of ERK-5 activity.

28. A method of antagonizing ERK-5 associated activity in a mammal comprising administering to said
25 mammal an antagonist to ERK-5 in an amount sufficient to effect said antagonism.

29. A method of agonizing ERK-5 associated activity in a mammal comprising administering to said mammal an agonist to ERK-5 in an amount sufficient to effect said
30 agonism.

30. A method of treating diabetes mellitus, skeletal muscle diseases, Alzheimer's disease, or peripheral neuropathies in a mammal with an agonist or antagonist of ERK-5 activity comprising administering the agonist or
5 antagonist to a mammal in an amount sufficient to agonize or antagonize ERK-5 associated functions.

31. A pharmaceutical composition comprising an ERK-5 agonist or antagonist in an amount sufficient to alter ERK-5 associated activity, and a pharmaceutically
10 acceptable diluent, carrier, or excipient.

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FIG. 1a.

1 ggctctgcggggtgggcagctcccgggctgcatgagctctccgcgcccggxggcagt 60
 -----+-----+-----+-----+-----+-----+
 ccgagacgccccacccgtcgagggcccgacggtactcgagaggcggcggccxcccgtca
 M S S P P P G G S -
 61 ggcttttaccgccaggaggtgaccaagacggcctgggaggtgcgcgccgtgtaccgggac 120
 -----+-----+-----+-----+-----+-----+
 ccgaaaatggcggctcctccactggttctgccggaccctccacgcgcggcacatggccctg
 a G F Y R Q E V T K T A W E V R A V Y R D -
 121 ctgcagcccgtgggctcgggcgcctacggcgcggtgtgctcggccgtggacggccgcacc 180
 -----+-----+-----+-----+-----+-----+
 gacgtcgggcaccccgagcccgcggtgccgcgccacacgagccggcacctgccggcgtgg
 a L Q P V G S G A Y G A V C S A V D G R T -
 181 ggcgctaaggttgccatcaagaagctgtatcgcccttccagtccgagctgttcgccaag 240
 -----+-----+-----+-----+-----+-----+
 ccgcgattccaacggtagttcttcgacatagccgggaaggctcaggctcgacaagcggttc
 a G A K V A I K K L Y R P F Q S E L F A K -
 241 ctgcctaccgcgagctgcgcctgtcaagcacatgcgccacgagaacgtgatcgggctg 300
 -----+-----+-----+-----+-----+-----+
 gagcggatggcgcctcgacgcggacgagttcgtgtacgcgggtgctcttgactagcccgc
 a L A Y R E L R L L K H M R H E N V I G L -
 301 ctggacgtattcactcctgatgagaccctggatgacttcacggacttttacctggtgatg 360
 -----+-----+-----+-----+-----+-----+
 gacctgcataagtgaggactactctgggacctactgaagtgcctgaaaatggaccactac
 a L D V F T P D E T L D D F T D F Y L V M -
 361 ccgttcatgggcaccgacctgggcaagctcatgaaacatgagaagctaggcgaggaccgg 420
 -----+-----+-----+-----+-----+-----+
 ggcaagtaccctggctggaccctcgagtactttgtactcttcgatccgctcctggcc
 a P F M G T D L G K L M K H E K L G E D R -
 421 atccagttcctcgtgtaccagatgatgaaggggctgaggtatatccacgctgccggcatc 480
 -----+-----+-----+-----+-----+-----+
 taggtcaaggagcacatggtctactacttccccgactccatataggtgcgacggccgtag
 a I Q F L V Y Q M M K G L R Y I H A A G I -
 481 atccacagagacctgaagcccggaacctggctgtgaacgaagactgtgagctgaagatc 540
 -----+-----+-----+-----+-----+-----+
 taggtgtctctggacttcgggcccgttgaccgacacttgcttctgacactcgacttctag
 a I H R D L K P G N L A V N E D C E L K I -

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FIG. 1b.

541 ctggacttcggcctggccaggcaggcagacagtgagatgactgggtacgtggtgacccgg
-----+-----+-----+-----+-----+-----+ 600
gacctgaagccggaccggtccgtctgtcactctactgacccatgcaccactgggcc
0 L D F G L A R Q A D S E M T G Y V V T R -
601 tggtagccgggctcccagaggtcatcttgaattggatcggtacacgcagacgggtggacatc
-----+-----+-----+-----+-----+-----+ 660
accatggccccgagggtccagtagaacttaacctagcgcatgtgctgtgccacctgtag
0 W Y R A P E V I L N W I A Y T Q T V D I -
661 tggctgtgggctgcatcatggcggagatgatcacaggcaagacgctgttcaagggcagc
-----+-----+-----+-----+-----+-----+ 720
accagacacccgacgtagtagccgctctactagtgtccgttctgcgacaagttcccgtcg
0 W S V G C I M A E M I T G K T L F K G S -
721 gaccacctggaccagctgaaggagatcatgaaggtgacggggacgcctccggctgagttt
-----+-----+-----+-----+-----+-----+ 780
ctggtggacctggtcgacttctctagtacttccactgcccctgaggaggccgactcaaa
0 D H L D Q L K E I M K V T G T P P A E F -
781 gtgcagcggctgcagagcgatgaggccaagaactacatgaagggcctccccgaattggag
-----+-----+-----+-----+-----+-----+ 840
cacgtcgccgacgtctcgctactccggttcttgatgtacttcccggaggggcttaacctc
0 V Q R L Q S D E A K N Y M K G L P E L E -
841 aagaaggattttgcctctatctgaccaatgcaagccctctggctgtgaacctcctggag
-----+-----+-----+-----+-----+-----+ 900
ttcttcctaaaacggagataggactggttacgttcgggagaccgacacttggaggacctc
0 K K D F A S I L T N A S P L A V N L L E -
901 aagatgctggtgctggfcgaggacatcaggttgactgcaggcgagtttctttcccatccc
-----+-----+-----+-----+-----+-----+ 960
ttctacgaccacgacctgcgctgtagtccaactgacgtccgctcaaagaaagggtaggg
0 K M L V L D A D I R L T A G E F L S H P -
961 tacttcgagtccttgacgacacggaagatgagccccagggtccagaagtatgatgactcc
-----+-----+-----+-----+-----+-----+ 1020
atgaagctcagggacgtgctgtgccttctactcggggtccagggtcttcatactactgagg
0 Y F E S L H D T E D E P Q V Q K Y D D S -
1021 ttgactactttgaccgcacactggatgaatggaagccgtgttacttacaaagaggtgct
-----+-----+-----+-----+-----+-----+ 1080
aaactgatgaaactggcgtgtgacctacttaccttcgggcacaatgaatgtttctccacga
0 F D Y F D R T L D E W K P C Y L Q R G A -

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FIG. 1c.

1081 cagcttcaagcctccccggcagctggggggccagggtctccaaggagacgcctctgtgaag
-----+-----+-----+-----+-----+-----+ 1140
gtcgaagttcggaggggcccgtcgacccccgggtcccagagggtcctctgcggagacacttc
Q L Q A S P A A G G Q G L Q G D A S V K -
1141 atctctgggctccgggggtggcagtgaggaccaccttcaccttcacctgagaggggactc
-----+-----+-----+-----+-----+-----+ 1200
tagagacccgaggccccaccgtcactcctggtggaagtggaagggtggactctcccctgag
I S G L R G G S E D H L H L P P E R G L -
1201 tcgttgccaccttgaccttggctggggcttgcacccaaggcatccatcagagcagacgc
-----+-----+-----+-----+-----+-----+ 1260
agcaacggtggaactggaaccgaccccgaaacgtagggtccgtaggtagtctcgctctgcg
S L P P *

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FIG. 2.

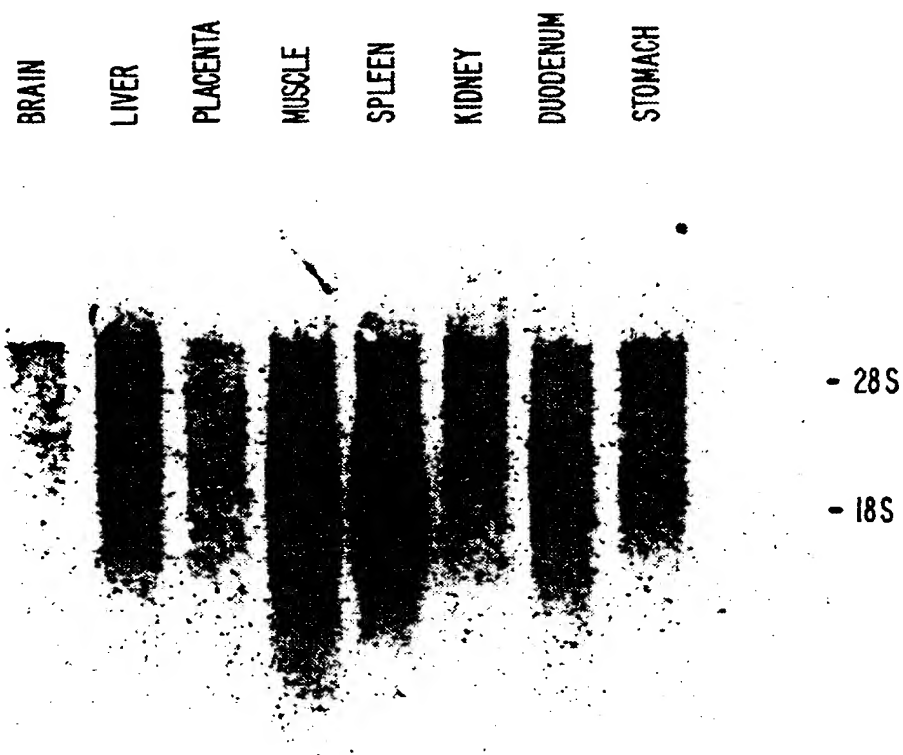


FIG. 3.

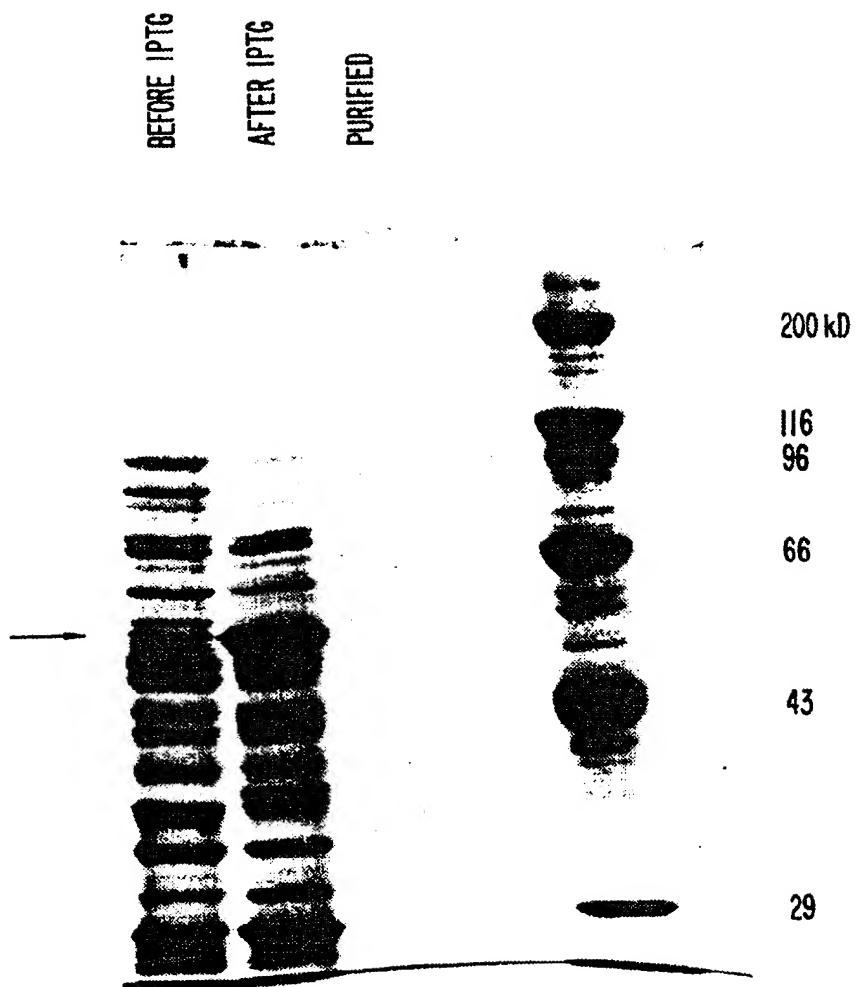
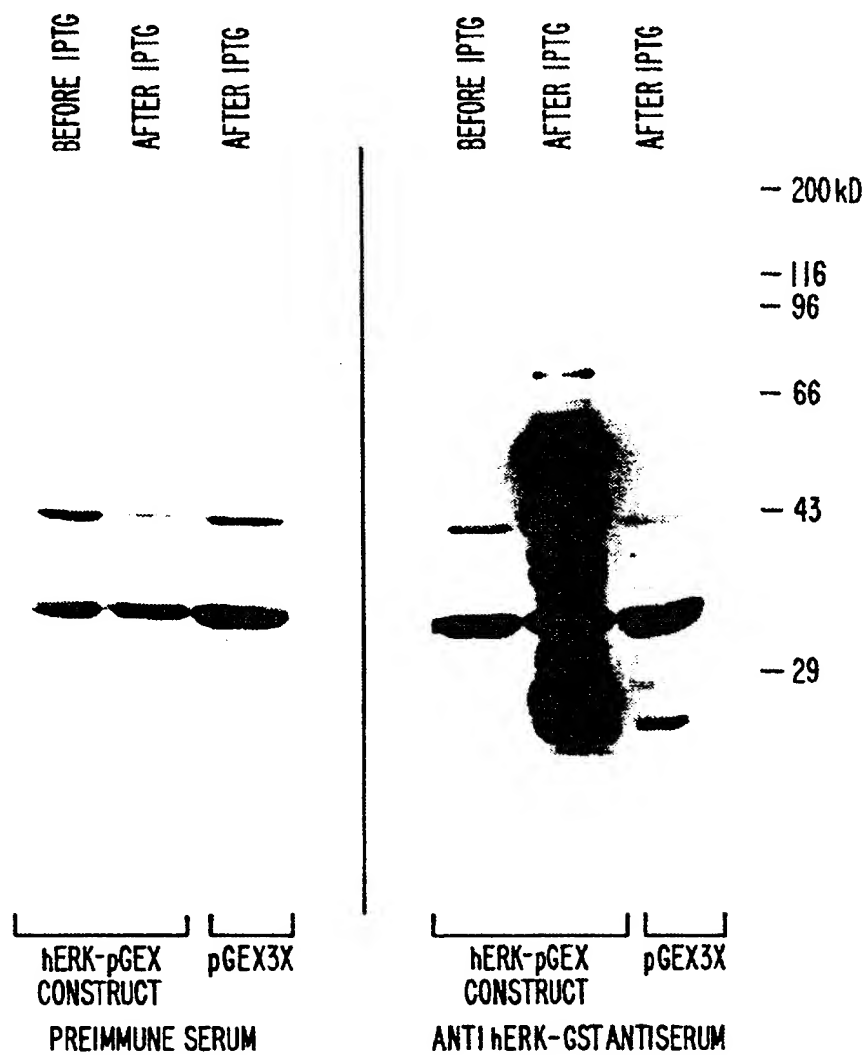
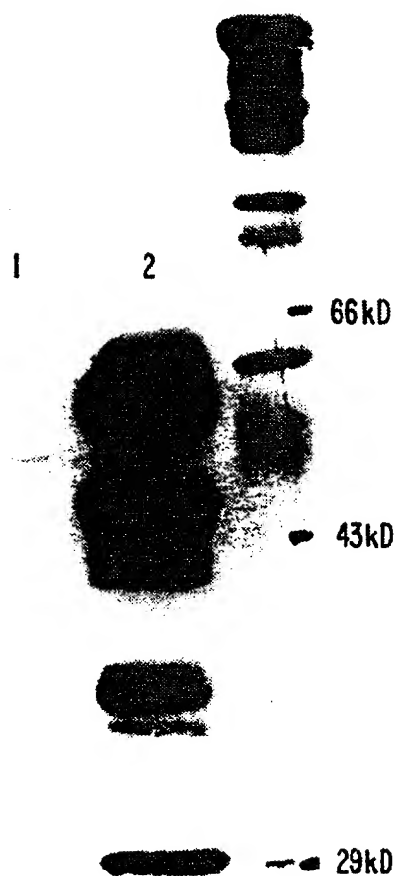


FIG. 4.



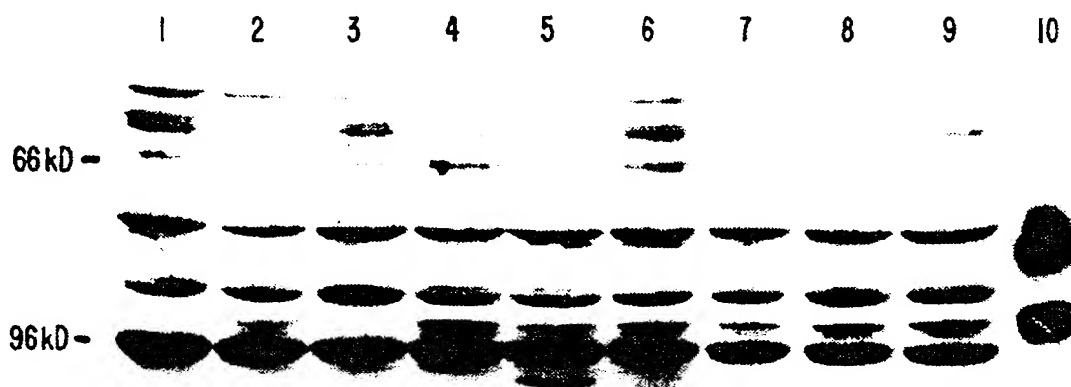
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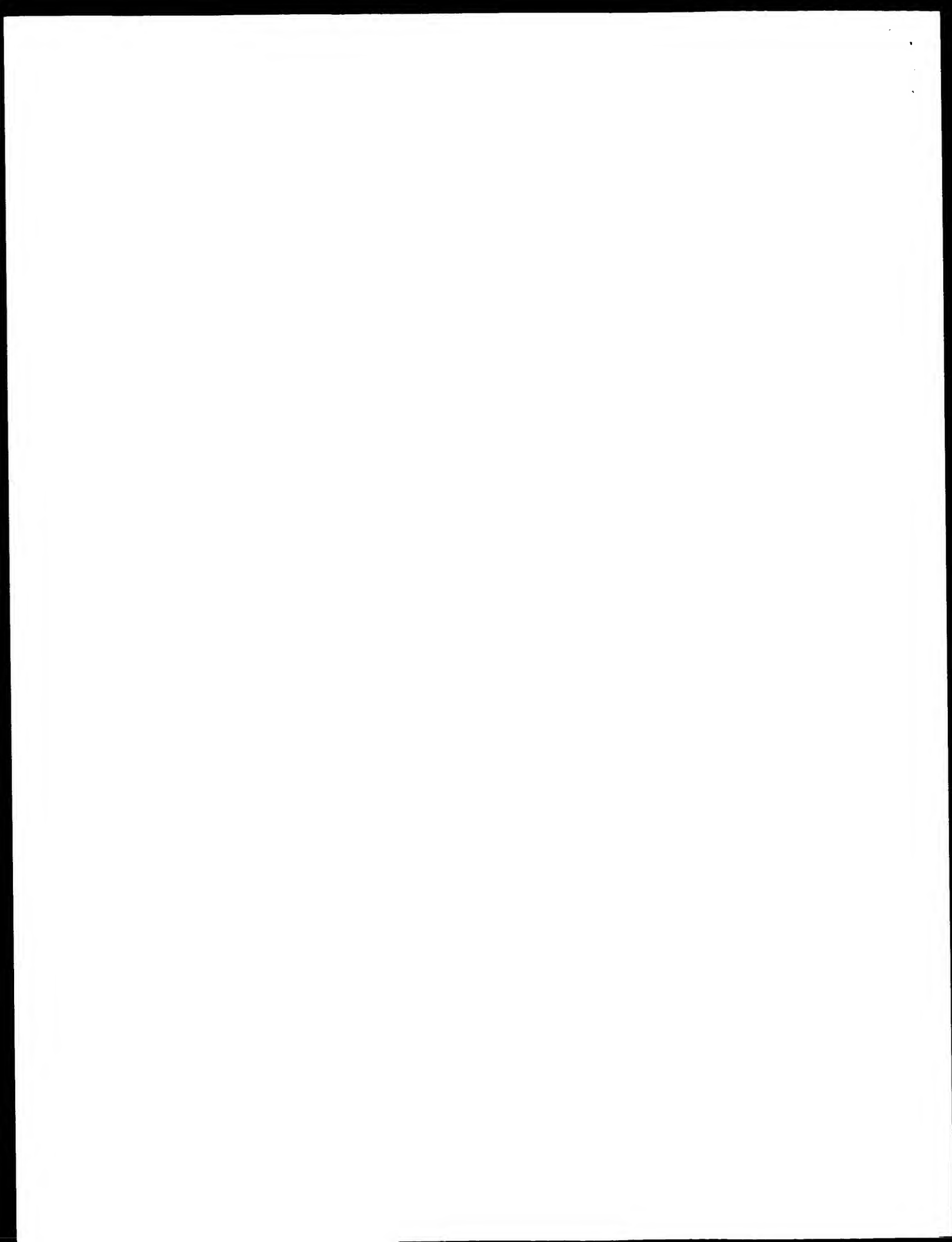
FIG. 5.



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*FIG. 6.*





INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵: C12N 15/54, 9/12, C12Q 1/68 C12N 5/10, C12P 21/08, G01N 33/53 C12N 5/20, C12Q 1/48, A61K 37/02	A3	(11) International Publication Number: WO 94/21781 (43) International Publication Date: 29 September 1994 (29.09.94)
(21) International Application Number: PCT/IB94/00089 (22) International Filing Date: 18 March 1994 (18.03.94) (30) Priority Data: 08/029,404 19 March 1993 (19.03.93) US (71) Applicant: MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN E.V. (DE/DE); Residenzstrasse 1A, D-8000 München 2 (DE). (72) Inventors: LECHNER, Cornelia; Spitzelbergstrasse 5D, D- 8000 München 71 (DE). MOLLER, Neils, Peter, H.; Heiterwanger Strasse 32, 5D, D-8000 München 70 (DE). ULLRICH, Axel; Adalbertstrasse 108, D-8000 München 40 (DE). (74) Agent: WEICKMANN, H.; Kopernikusstrasse 9, D-81679 München (DE).		(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KG, KP, KR, KZ, LK, LU, LV, MD, MG, MN, MW, NL, NO, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i> (88) Date of publication of the international search report: 10 November 1994 (10.11.94)
(54) Title: EXTRACELLULAR SIGNAL-REGULATED KINASE, SEQUENCES, AND METHODS OF PRODUCTION AND USE (57) Abstract <p>The present invention relates, in general, to an extracellular signal regulated kinase, ERK-5. In particular, the present invention relates to nucleic acid molecules coding for ERK-5; ERK-5 polypeptides; recombinant nucleic acid molecules; cells containing the recombinant nucleic acid molecules; antisense ERK-5 nucleic acid constructs; antibodies having binding affinity to an ERK-5 polypeptide; hybridomas containing the antibodies; nucleic acid probes for the detection of ERK-5 nucleic acid; a method of detecting ERK-5 nucleic acid or polypeptide in a sample; kits containing nucleic acid probes or antibodies; a method of detecting a compound capable of binding to ERK-5 or a fragment thereof; a method of detecting an agonist or antagonist of ERK-5 activity; a method of agonizing or antagonizing ERK-5 associated activity in a mammal; a method of treating diabetes mellitus, skeletal muscle diseases, Alzheimer's disease, or peripheral neuropathies in a mammal with an agonist or antagonist of ERK-5 activity; and a pharmaceutical composition comprising an ERK-5 agonist or antagonist.</p>		

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A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C12N15/54 C12N9/12 C12Q1/68 C12N5/10 C12P21/08
G01N33/53 C12N5/20 C12Q1/48 A61K37/02

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

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A	WO,A,91 19008 (REGENERON PHARMACEUTICALS INC.) 12 December 1991 ---	1-31
A	SCIENCE vol. 249 , 6 July 1990 pages 64 - 67 BOULTON, T.G. ET AL. 'An insulin-stimulated protein kinase similar to yeast kinases involved in cell cycle control' ---	1-31
A	BIOCHEM. BIOPHYS. RES. COMM. vol. 182, no. 3 , 14 February 1992 pages 1416 - 1422 OWAKI, H. ET AL. 'Extracellular signal-regulated kinases in T cell characterization of human ERK1 and ERK2 cDNAs' --- -/--	1-31

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Date of the actual completion of the international search

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A	<p>CELL vol. 65 , 17 May 1991 pages 663 - 675 BOULTON, T.G. ET AL. 'ERKs: A family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF' -----</p>	1-31

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